RESPONSES OF ROSTRAL HYPOTHALAMIC NEURONES TO PERIPHERAL TEMPERATURE AND SOME PUTATIVE THERMOREGULATORY AGENTS

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PAUL SWEATMAN

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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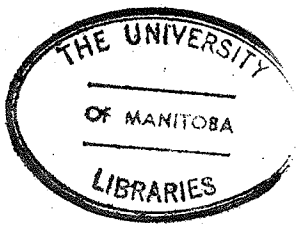

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ABSTRACT

Spontaneously firing rostral hypothalamic cells of the methoxyflurane anesthetized cat were tested for their response to peripheral thermal stimulation and microiontophoresed noradrenaline, serotonin, prostaglandin E_1, dopamine and histamine, drugs thought to be involved in thermoregulation. Nineteen of 240 (7.9%) cells tested were responsive to peripheral thermal stimulation. Fourteen were cold responsive and 5 were warm responsive. Forty-five of 90 (50%) cells tested responded to noradrenaline; forty-one of 98 (41.6%) cells tested responded to serotonin; 11 of 88 (12.5%) cells tested responded to prostaglandin E_1; 36 of 89 (40.4%) cells tested responded to DA; 25 of 95 (26.3%) cells tested responded to HA. Depression was the predominant effect. The findings for noradrenaline and serotonin are in general agreement with previous work while those for prostaglandin E_1 are not.

Dopamine and histamine have never before been iontophoresed on hypothalamic cells identified by their responsiveness to peripheral thermal stimulation. Thermoresponsive neurones were significantly more likely to respond to and be depressed by dopamine than nonthermoresponsive neurones. No such relationship was found for histamine but, as with dopamine, the results can be used as an explanation at the neuronal level of the apparent involvement of these two agents in thermoregulation.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION AND RATIONALE

The hypothalamus is the most important central nervous system structure associated with the regulation of body temperature. The ability to thermoregulate disappears when the brain is sectioned below the hypothalamus but is unimpaired when sectioned above the hypothalamus (Keller and Hare, 1932, see Bligh, 1973). Within the hypothalamus there is evidence for separate structures being responsible for heat production and heat loss.

Lesion and stimulation studies have confirmed that the regions hypothalamus anterior and regio preoptica (both hereafter referred to as the POAH rostral hypathalamus) contain the principle neural substrate for the control of responses to heat stress, and that the hypothalamus posterior is principally concerned with the control of responses to cold stress (See Bligh, 1973). There is reciprocal interactions of the two regions such that, for example, during heat stress, the heat loss system in the rostral area is activated with subsequent inhibition of the heat gain system in the posterior area. The actual neural circuitry involved is not known but findings from single unit studies indicate the existence of populations of neurones specifically involved in heat production, heat loss and reciprocal inhibition of both (see Bligh, 1973).

From these findings neuronal models of thermoregulation have been proposed, a composite of which is shown in Figure 1. Concominant with the development of these models was speculation on what compounds (neurohumors, modulators, mediators, hormones, neurotransmitters) mediated the excitatory and inhibitory effects indicated in Figure 1. In the early 1960's the catecholamines, adrenaline and noradrenaline and the indolamine, serotonin (5-hydroxytryptamine) were shown to affect body temperature and it was suggested they did so by some "influence on the setting mechanism" (VonEuler, 1961). However, an actual mechanism and site were not proposed until the work of (Feldberg and Myers, 1964, 65) who demonstrated an antagonistic effect between 5-HT and the catecholamines on cat rectal temperature; the effects appeared to be mediated by the rostral hypothalamus. The neuronal models were subsequently modified to incorporate these findings and have proven useful constructs in understanding how a drug might influence body temperature through an action on thermoregulatory neuronal pathways.

For example, activation of heat loss mechanisms and/or deactivation of heat production mechanisms is a reasonable explanation of how a thermolytic agent such as noradrenaline(NA) brings about a fall in core temperature. Relating this to the neuronal model in Figure 1 centrally injected NA presumably diffuses to enough sensitive sites on neurones such that there would be an activation of the neurones in the heat loss pathway (upper pathway in Figure 1) and by way of the crossed inhibitory pathway, deactivation of the neurones in the heat production pathway(lower pathways in Figure 2) with the resultant fall in temperatures indicated by the effector mechanisms e.g. panting, shivering, etc. By iontophoresing NA on cells identified by their response to thermalstimulation, one should be able to confirm such a proposed mechanism of action for the thermolytic action of NA. That is, if a cell is encountered that is excited by warming (be it from a peripheral, spinal or hypthalamic source) it is assumed this cell lies in the heat loss pathway (or the crossed inhibitory pathway) and one would expect that if it were responsive to NA, that if NA is in fact involved, it would be excited. The reverse finding might also be expected for a cold excited cell. For a thermogenic agent, the opposite relationships would be expected. That is 5-HT should increase the discharge rate of cold excited cells and depress the rate of warm excited cells.

Therefore iontophoresis would appear to be an excellent tool for testing the validity of the thermoregulatory neuronal models and giving an indication of the possible mechanism of action of agents thought to be involved in thermoregulation. Much of this testing has already been done and the results have been confusing. Some workers have found the predictable relationships (Hori and Nakayama, 1973) while others have formed a total lack of correlation between thermal response pattern and drug sensitivity (Jell, 1973) However, Jell and Sweatman(1977) recently formed relationships which did not depend on the directionality of response to thermal or drug stimulation. Rostral hypothalamic cells responding to peripheral thermal stimulation tended to be responsive to the highly potent thermogenic drug prostaglandin E (P.G.E.). Futhermore P.G.E. sensitive cells were more likely to be NA and 5-HT sensitive than non-P.G.E. sensitive cells, indicating possible common targets of action for 5-HT and NA. (discussed in more detail later) While the significance of such findings are less clear than, say, NA excitation of warm excited cells, they do establish a further usefulness of iontophoresis as a technique for the investigation of agents possibly involved in temperature regulation.

Iontophoresis then, can lend supportive evidence to other pharmacological evidence for any putative thermoregulatory agent. Correlation with thermoresponsive neurones can be looked for, both in terms of predicted direction of response and of a simple correlation with thermoresponsive cells, and correlations among multiple drug responses can also be looked for.

While this study was designed as a drug study, primarily, microelectrode recordings from single cells also facilitate comment on the incidence and response patterns of various cells types. We will be identifing cells by their response or lack of response to peripheral (and some local) thermalstimulation. These findings will be discussed in terms of those of other workers and how they reflect on the functioning of the hypothalamic thermoregulatory system.

The study reported here concerns five drugs which have received considerable attention as being involved in hypothalamic control of body temperature. These agents are noradrenaline (NA), 5-hydroxytryptamine (5-HT), prostaglandin E_1 (P.G.E_1), dopamine (DA) and histamine (HA). The evidence for their involvement is of two types:
they have centrally mediated temperature effects and they exhibit an endogenous presence consistant with a neurophysiological role. These lines of evidence will be discussed as neuropharmacological and neuro anotômical evidence respectively and one largely confined to work done in the cat. It was decided the evidence from the cat alone was sufficient to justify the study described here. However for some areas evidence from other species is included and for a complete discussion the reader should see the review by Hellon (1975).

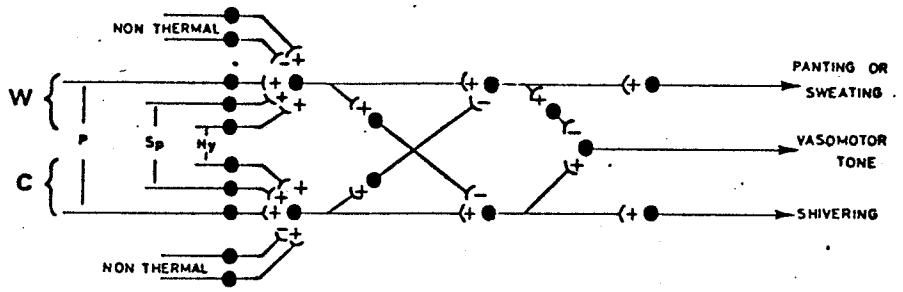


FIGURE 1. Neuronal model of thermoregulation (modified from Bligh, 1973). 'W' and 'C' reter to warm and cold temperature information inputs from the periphery (P), the spinal cord (Sp), and the hypothalamus (Hy) converging on two hypothalamic neurones.

Evidence for the involvement of NA, 5-HT, P.G.E., DA, and HA in temperature regulation.

NA

Feldberg and Myers (1964) were the first to demonstrate the hypothermic effect of centrally administered NA in the unanesthetized cat. At room temperature, NA caused a fall of 1-2°c in rectal temperature when injected into the lateral ventricle. An intracerebroventricular (ICV) NA injection could also abolish pyrogen-induced shivering and fever resulting in a fall in temperature. The hypothermias were associated with vasodilitation as evidenced by a rise in pinna temperature.

It is likely that the ICV-injected NA brings about hypothermia by an action on the rostral hypothalamus. The drug probably diffuses into the medial border in the third ventricle as it is carried past in the cerebral spinal fluid (C.S.F.). Support for this comes from the demonstration that microinjection of NA into the rostral hypothalamus evokes hypothermia similar in nature to that induced by NA injected but microinjections into the posterior or ventromedial hypothalamus are ineffective (Feldberg and Myers, 1965). Similar findings were obtained by Rudy and Wolf (1971).

Release studies (Myers and Chinn, 1973) also support a role for NA activation of heat loss pathways in the cat rostral hypothalamus. Increased NA release occurred only at high ambient temperatures and only from the rostromedial hypothalamus.

The use of pharmacological agents affecting endogenous stores of NA has also resulted in support for NA mediating hypothermia in the cat hypothalamus. ICV injections of imipramine and desipramine which inhibit uptake of NA from the synaptic cleft and should therefore potentiate it's effect, resulted in a fall in body temperature (Cranston et al., 1972). Kennedy and Burks (1972) injected tyramine, an indirect sympathomimetic amine into the lateral ventricles of cats and observed a hypothermia which could not be repeated when endogenous stores of catecholamines

were depleted with 6-hydroxydopamine or reserpine. Subsequent injection of NA, however, did produce the same hypothermia indicating NA receptors mediating the hypothermia were still intact.

Using the histofluorescence technique, Fuxe(1965) demonstrated the existence of monoamine terminals in many parts of the rate C.N.S. A high density of NA terminals was observed in many hypothalamic nuclei including the preoptic area with the anterior hypothalamus showing a low density of NA terminals . A similar distribution was seen in human fetus (Nobin and Bjorklund, 1973). Poitras and Parent (1975) have demonstrated catecholamine (CA) containing neurones and axon terminals were seen in the preoptic and anterior hypothalamus.

NA has been quantitatively assessed in all rat hypothalamic nuclei (Palkovits et al, 1974) and the hypothalamus and area postrema have the highest NA concentration of any brain region in cats and dogs (See review by Hellon, 1975).

Apparently most of the NA containing cell bodies of the C.N.S. lie within the brainstem nuclei, the locus coeruleus (Dahstrom and Fuxe, 1964). Most of the hypothalamic nuclei probably receive locus coeruleus projections (Fuxe, 1965). Using neuroanatomical and electrophysiological techniques, McBride and Sutin (1976) in the cat have obtained highly suggestive evidence for projections to the preoptic (and lateral) hypothalamus.

In summary, there is good evidence for the involvement of NA in hypothalamic control of body temperature. Pharmacological experiments indicate a consistant hypothermic action and the neuroanatomical evidence suggests release of NA in the hypothalamus results from locus coeruleus activation. In fact, Eiserman (1973) reports electricalstimulation of brainstem nuclei including the locus coeruleus can affect the firing rate of assumed thermoregulatory neurones in the cat hypothalamus.

5-HT

The evidence from ICV and microinjection studies in the cat favours a hyperthermic role for 5-HT. Feldberg and Myers (1964) original demonstration of a raise in body temperature to 5-HT injected ICV was challenged by Kulkarni (1967) who observed a primary fall in body tempenature followed by a rise. However,

his techniques were not identical to those of Feldberg and Myers. Banerjee et al., (1968) confirmed both Feldberg's and Kulkarni's findings and suggested that ICV " 5-HT raises' rectal temperature in the cat when the amount is not too large and that a hypothermic effect when it occurs results from a paralysis of cells in the anterior hypothalamus which are excited by small doses."

There has been no dispute or confirmation of the effect of intra-hypothalamic injection of 5-HT which causes a biphasic rise in rectal temperature, associated with shivering, vasoconstriction and increase in respiration rate (Feldberg and Myers, 1965). Except for the change in respiration rate, the hyper-thermia is associated with the same obserable effect on mechanisms whether 5-HT is administered ICV or directly into the rostrol hypothalamus.

Another approach which implicated 5-HT as having a central hyper-thermic role in the cat was the perfusion of 5-hydroxytryptophan (5-HTP) into the third ventricle, the anterior horn or the inferior horn of the left lateral ventricle of the cat (El Hawary and Feldberg, 1966). 5-HTP is the precursor of 5-HT. As the concentration of perfused 5-HTP increased, so did the output of endogenous 5-HT in the effluent collected from the aqueduct. Shivering and a variable increase in rectal temperature was observed only when the third ventricle was perfused with 5-HTP indicating involvement of the hypothalamus.

Use of the monoamine oxidase inhibitor, tranylcypromine, offers further evidence for the hyperthermic role of endogenous 5-HT. Perfusion of the third ventricle with tranylcypromine resulted in an increase in effluent 5-HT plus a temperature rise associated with shivering (El Hawary, Feldberg and Lolli, 1967). Tranylcypromine could abolish or reverse the hypothermia induced by sodium pento-barbitone or chloralose anesthesia (E l Hawary, Feldberg and Lotti, 1967, Feldberg and Lotti, 1967), as well as result in an increase in 5-HT output.

Dahlstom and Fuxe (1964) showed in the rat that most of the 5-HT containing cell bodies lie in the raphe nuclei of the brainstem. Apparently this is common to all mammals (Hellon 1975) including the cat where in addition some 5-HT containing cell bodies were found in the lateral area of the middle and mamillary hypothalamic regions (Poitras and Parent 1975).

Histochemical, autoradiographic, lesion experiments, release studies and electrophysiological studies all support the notion that 5-HT containing cell bodies of the raphe project to the rostral hypothalamus (among many other areas) and are involved in thermal homeostasis. Axon terminals containing 5-HT were seen in most parts of the rat hypothalamus (Dahlstrom and Fuxe, 1964). Using autoradiography, Pierce et al (1976) demonstrated in the cat a raphe projection to the hypothalamus, confirmed by Bobillier et al. (1976) who further found projections to the rostral hypothalamus from the central superior, dorsal, magnus and pontine raphe. Pasquier et al (1976) found decreased 5-HT levels in the hypothalamus and preoptic regions following lesions in the central superior nucleus of the cat. Upon electrical stimulation of the raphe an increase in C.S.F. 5-HT levels collected from the third ventricles of cats (Ashkenazi et al, 1973) suggests that increased 5-HT release in the hypothalamus is associated with the increased discharge of raphe neurones. Also in the cat, raphe stimulation can alter the firing rate of rostral hypothalamic neurones responsive to local temperature change (Eisenman, 1973). The raphe itself has cells responsive to both local (Cronin and Baker, 1976) and peripheral (Jahn's 1976) temperature changes in the cat and rat respectively, although Jahn's findings have been disputed (Tralson and Jacobs, 1976). The role of 5-HT in the rat is particularily confusing once ICV injected 5-HT causes a fall in temperature where as intrahypothalamically injected 5-HT results in a rise as does raphe stimulation (Hellon, 1975).

In summary then the pharmacological and anatomical evidence is suggestive of a role for 5-HT in temperature regulation. It appears 5-HT release in the hypothalamus (possibly as a result of raphe activation) results in hyperthermia.

Prostaglandin E

P.G.s of the 'E' series have a powerful hyperthermic action after ICV injection in the unanesthelized cat (Milton and Wendlandt, 1971). The site of action appears to be the rostral hypothalamus (Cooper and Veale, 1975).

P.G. E. has been implicated in fever. It is thermogenic in all animals yet tested (except in the echidna, see Hellon, 1975), and an increase in P.G.E. - like activity in C.S.F. can be readily associated with a bacterial pyrogen induced fever (Feldberg and Gupta, 1973, Feldberg et al, 1973). Further,

leucocyte pyrogen endogenous pyrogen and P.G.E. all appear to elicit fever by acting on the rostral hypothalamus (Veale, et al, 1976).

The notion that P.G.E. mediates bacterial pyrogen fever was further supported when antipyretic drugs known to inhibit P.G.E. synthesis could abolish the fever and elevated P.G.E. -like activity in C.S.F. of cats which were apparently caused by intravenous administration of bacterial pyrogen (Feldberg et al., 1973). Findings in the rabbit on the other hand do not support the proposed role of an involvement of P.G.E. in pyrogen fever (Veale et al., 1970).

However, both pyrogen and P.G.E. fever appear to involve a 5-HT synapse. Cats pretreated with a presumed 5-HT blocker would not develop a fever when bacterial pyrogen was given I.V. in doses which normally induce a fever associated with shivering. Using a 5-HT depletor, there was suppression of shivering and reduction of the fever response to I.C.V. administrating of pyrogen or P.G.E. (Milton and Harvey, 1975). These findings indicate only that the fever response of P.G.E. might involve a 5-HT synapse in the heat gain pathway involving shivering. (As stated earlier, the involvement of P.G.E. in physiologic bacterial-pyrogen-induced fever is still an open question).

Of interest and of possible importance here is the evidence of Ford (1974) and Jell and Sweatman (1977) of a relationship between thermo-responsive neurons and P.G.E. sensitive neurons of which more is said in the discussion.

Studies on endogenous P.G.E. have offered inconclusive evidence for a neurophysiological role for P.G.E. There are no demonstrable P.G. tracts and P.G. cannot be localized to nerve terminals as it is not stored but apparently synthesized on demand (Cocean, 1974). P.G.'s of the E, and F series occur in the C.N.S. of various species including the cat and more may be associated with grey matter than white (Holmes and Horton, 1968). However, true determination of P.G. levels in tissue is difficult or impossible since almost any stimulus including dissection etc., can cause synthesis. In spite of this, there does appear to be a correlation with increased P.G. synthesis and release associated with increased neural activity as evidenced by their presence in cortical per-

fusates, spinal cord washings and effluents form cerebral ventricles.

Thermoregulation offers some evidence from endogenous P.G.E. studies for a role of P.G.E., Feldberg et al., (1973) showed increased P.G.E. - like activity in the cerebrospinal fluid (C.S.F.) of cats with a bacterial pyrogen induced fever. Using antipyretic drugs known to inhibit P.G. synthesis a reduction in fever and P.G.E.- like activity was found. A similar involvement of P.G.E. and leucocyte pyrogen was not found in the rabbit (Cranston et al, 1976). Veale et al., (1976) could find no difference in P.G. levels of perfusates of PoAH tissue in febrile and nonfebrile rabbits. Similarily, Beleslin and Myers (1971) using the monkey, observed the presence of a P.G.- like substance in perfusates from various brain areas including the hypothalamus and there was no correlation with fever

But results from the dog, like the cat, are slightly suggestive of a role for P.G.E.. I.C.V. injected 5-HT causes a rise in temperature as well as stimulates P.G.E. release where as the catecholamines do not. The results from the cat and dog are suggestive of a mediator role for P.G. E.

In summary, P.G.E. is a potent thermogenic agent and although the evidence is inconclusive, P.G.E. has been implicated in the mediation of fever and in the response to cold. The neuroanatomical and chemical evidence while far from definitive are not inconsistant with the involvement of P.G.E. in thermoregulation.

DA

DA has a thermolytic effect on cat body temperature when injected centrally. I.C.V. administration of DA causes a fall in rectal temperature in cats (Kennedy and Burks, 1974) which was associated with vasodilatation (increase in ear temperature and flushing the nasal skin) and reduced motor activity. Haloperidol, a presumed DA receptor blocker, significantly reduced the hypothermic effect of DA but had no affect on the DA induced behavioural changes indicating this DA hypothermia to be the result of an action on thermoregulatory

effector mechanisms and no an incidental result of an action on behaviour.

Intrahypothalamic DA injection induced in the cat a hypothermia which was associated with reduced motor activity but no apparent change in ear temperature or respiratory rate(Quock and Gale, 1974), Quock, personal communication). The hypothermia could be attenuated by systemic administration of haloperidol and abolished by intrahypothalamic administration of haloperidol.

It appears, then, that DA may be activating heat loss mechanisms and suppressing heat producing mechanisms when injected directly into the rostral hypothalamus.

Fuxe (1964-65) demonstration the presence of catecholamine terminals in virtually all rat hypothalamic nuclei visualized. The presence of DA throughout the rat hypothalamus has once been confirmed (Fuxe et all, 1974, Pal Kovits et al, 1974, Versteeg et al, 1976).

Some of the projections of the dopaminergic system have been elucidated while some only inferred. The discussion will be limited to evidence of DA neurones associated with the rostral hypothalamus. Pal Kovits et al, (1974) demonstrated DA in relatively low concentrations in the preoptic suprachiasmatic nucleus and the anterior hypothalamus (2:1 distribution) of the rat. Their technique did not allow them to say whether this DA was in terminals or cell bodies. Bjorklund et al. (1975) gave strong evidence of DA fibers system in the medial preoptic area, periventricular and the suprachiasmatic preoptic nuclei. The termination of some of these DA fibers in the mentioned hypothalamic nuclei is probable although actual DA terminals were not demonstrated. Kizer et al (1976) gave evidence of another DA projection to the preoptic region in the rat. Lesions in the zona compacta, known to contain DA cell bodies, resulted in lowered DA levels in all seven preoptic hypothalamic nuclei assayed. However, only the values for the suprachiasmatic nucleus and the medianeminence could be considered statistically significant.

A recent study in the cat (Poitras and Parent, 1975) has demonstrated the presence of CA neurones in cat hypothalamic region. Both CA cell bodies and terminals were seen in the preoptic and anterior hypothalamic regions. It is unknown how may of these CA neurones were DA neurones but the finding of homovanillic acid, the main metabolite of DA, in the cat hypothalamus (Poirier and Sourkes, 1976) supports the notions of an active dopaminergic system in cat hypothalamus.

To summarize then, central injection ofDA in the cat evokes a fall in temperature. This, plus the observed dopaminergic systems in the hypothalamus are strong indicators of an involvement of DA in the hypothalamic control of body temperature.

Histamine (HA)

The effect on cat body temperature of HA and some methylated congeners have only been investigated alter ICV injection (Clark and Cumby, 1976). HA caused a fall in temperature followed by a rise at doses of 100 mg or more. The initial hypothermia could be prevented by systemic pyrilamine, an H_1 receptor antagonist. The hypothermic phase was antagonized by central but not peripheral metiamide, an H_2 - receptors antagonist. 2-Methylhistamine, which is more active at H_1 - receptors than H_2 - receptors in some systems, caused an inital hypothermia followed by a hyperthermia. 4-Methylhistamine which acts primarily on H_2 receptors caused a delayed hyperthermia. (3-Methyhistamine, the amin metabotile of HA, had no effect on temperature).

ICV injected HA at 4°c ambient temperature produced the same degree of hypothermia as when administered at 22°c. (The hyperthermic phase was not studied). The hypothermic phase was reduced in magnitude at a high ambient temperature of30.5°c. Cats trained to escape an infrared heat source by lever pressing increase the amount of time spent pressing after ICV injection of HA which faciliated the development of hypothermia.

Clark and Cumby took little notice of the effector mechanisms with which the changes in temperature were associated. They did notice however, that tachyphoea was associated with the hypothermia.

HA has been demonstrated in brain in man (McGaer, 1964) monkey, rabbit, dog, cat and frog and in all cases its concentration is highest in the hypothalamus (Green 1970). Much of this HA is contained in mast cells which are numerous in the hypothalamus and which are known to store HA (Goth et al., 1976). However, a substantial proportion of brain HA is neural in origin. Subcellular fractionation studies in the rat show a significant amount of HA is present in the synaptosome, the pinched off nerve endings, and some can be identified as attached to synaptic vesicles (Schwartz, 1975).

Brownstein et al. (1974), using a sensitive enzymatic-isotope method, found a non-uniform distribution of HA in individual hypothalamic nuclei in the rat brain.

To date, no convincing demonstration of an histaminergic pathway has been made. However, there is a suggestion of one through the lateral hypothalamus in the medial forebrain bundle of the rat (See review by Schwartz, 1975).

In short, HA may be involved in thermoregulation. It is both thermolytic and thermogenic in the cat with apparently different receptors mediating each response. The central site of HA action, in the cat at least, has not been investigated. The neuroanatomical evidence is not inconsistant with it's proposed involvement.

Objectives

An iontophoretic study was conducted with the following objectives: to record from single rostral hypothalamic neurons in the cat and to attempt to identify them in terms of their responses to temperature stimuli; to study the sensitivity of recorded neurons to locally applied drugs thought to be involved in hypothalamic thermoregulation and to look for a possible relationship between drug sensitivity and thermal responsiveness as well as any correlations among multiple drug responses.

METHODS

Forty-four normothermic male and female cats (2.0 - 4.5 Kg) were used. In the first experiments anesthesia was induced using a halothane, nitrous oxide, oxygen mixture. Later nitrous oxide mixture was replaced in air as it gave no discernable advantage. Initially the cat was restrained in a 2' x 6" plexiglass tube and neck frame while the gas mixture was administered to the cat via a face mask. Induction was accomplished at a 4% halothane concentration in a 1.0 liter minute volume. As halothane anesthesia progresses the halothane concentration was lowered to about 2%. Once induced, the animal was removed from the tube and placed, on its back, on a heating pad. The face mask was taped to the face and a tracheostomy performed.

A T-shaped brass cannula was inserted into the trachea. One side of the cannula was attached to the anesthetic machine by means of a Tygon tube. The other side was connected to a 6' long tube free at the distal end. This tube served as a dead space to avoid the inhalation of room air. Expired air was analysed for carbondioxide by a Beckman LB1 CO_2 analyzer by sampling expired air from a third tube attached to the middle of the cannula.

Once the tracheostomy was performed, anesthesia was maintained with methoxyfluane in air at an initial concentration of 2% which was delivered from a second "copper kettle" vaporizer from the same Forreger gas anesthesia machine.

The femoral artery and vein were cannulated using polyethylene tubing and 3-way valves. Each cannula was previous filled with a solution of heparin 100 units of ml in lactated Kinger solution. The arterial cannula was connected to a Statham blood pressure transducer and blood pressure was recorded on a chart recorder. This gave an index of animal status anderanesthesia. Typically, light anesthesia could be maintained by reducing the inhaled anesthetic concentration; using blood pressure the presence of corneal reflex, and ear twitch as indices of depth. Absence of reflex, twitch, and low blood pressure indicated an excessive dept of anesthesia. The venouscannula was attached to a 5% dextrose in 0.9% saline solution drip which was administered at approximately 1 drip/min in order to replace body fluids and serve as an energy supply in long experiements (some experiments lasted over 40 hours).

The animal was placed in a Narishige stereotaxic frame and on a heating pad, the current of which was under feedback control via a rectal probe so that body temperature was maintained near 37-38°c . As a local anesthetic, xylocaine jelly was applied to the external auditory meatus before affixing the head with the ear bars. The ear and nose piece were then positioned and the scalp cut antero-posteriorly in the midline. Two holes were then made in the skull on either side of the midline at F 15 and the bone between them removed. The dura was reflected except just over the midline so that approximately 3 mm^2 area of brain was exposed on both sides of the midline.

Glass pipette electrodes were cleaned in dichromic acid and washed repeatedly in distilled water, then oven dried. Seven-barreled glass microelectrodes were constructed and pulled on a Narishige puller; their tips were broken back to approximately 5μm. The center recording barrel was filled with a 2 M NaCl solution and one of the side barrels with a 0.5 M NaCl solution to be used for current controls. The other five outside barrels were filled with one of the following solutions: 0.2 M 3,4 dihydroxyphenylethylamine HCl (DA), pH adjusted to 4 with NaOH; 0.2M histamine dihydrochloride, pH adjusted to 4 with NaOH; 0.2M L-norepinephrine bitartrate, pH adjusted to 5.5 with NaOH; 0.025M 5-hydroxytryptamine, creatine sulfate complex, pH adjusted to 5 with NaOH; 0.05M prostaglandin E_1, sodium salt pH7 as mixed. Electrode resistances ranged from 5 megohms to over 200 megohms.

The microelectrode was mounted on the moving stage of a Kopf 1207S stepping hydraulic microdriver. Electrode penetrations were directed toward the region of the rostral hypothalamus which lies between the anterior commissure and the optic chiasm, that is, between the frontal coordinates +14.0 and +16.0 and the vertical coordinates 0.0 and -5.0. Lateral displacement was 0.5 mm to 1.5 mm of the midline.

Only spontaneously firing cells were studies. When one was encountered, it was first tested for thermoresponsiveness and then for drug and current sensitivity. For some experiments if a cell did not respond to thermal stimulation it was not studied further.

Currents for electrophoretic drug applications were generated by a 6-channel, constant-current polarizer (Spencer, 1971). A reverse-polarity retaining current was automatically applied by the polarizer when not ejecting current. Retention currents between 10-15 n amps were used. Ejection currents used were between 50 - 150 nA. Extracellular action potentials, detected by the centre barrel, were amplified using an electrometer (WPI) and operational amplifier system, and bandpass-limited to the range 100 H2 to 20KH2. The amplified signals were then displayed on two oscilloscopes, one with fast sweep rate and the other slow, and fed into an amplitude discrimination circuit which produced a standard pulse every time the signal exceeded a preset level during an action potential. The sweep of the oscilloscope with the fast sweep rate was triggered from the action potential itself, and was used to check that the action potential amplitude did not drop below the preset discrimination level. Pulses from the discriminator were counted digitally over 1,2, or 3 second intervals before digital to analogue conversion and recording of the corresponding rate of firing of the neurone on a chart recorder. An event marker indicated periods of drug application.

For the most part peripheral thermal stimulation was accomplished by blowing warm (40°c) or cold (5.5°c) air into the cat's face. A domestic room air conditioner supplied the cold air and a domestic hair dryer the warm. The air flow was delivered directly into the face by means of flexible plastic hoses connected to the air sources and to the stereotaxic frame.

A cell was considered responsive to thermal stimulation, to a drug or to current only if the response was repeatable. Lack of response to a drug was considered to have occurred only when the cell responded to at least one other ejectant. If a cell responded in the same direction to both cooling and warming, it was not considered as thermoresponsive.

A cold responsive neurone was considered as one which showed an increase in discharge rate upon a cold stimulus or a decrease in rate upon warm stimulus. A warm responsive cell was considered to be a cell which responded to a warm stimulus with an increase in firing rate or with a decrease in rate upon a cold stimulus.

APPENDIXES TO METHODS.

1) Alternate method for induction of anesthesia

Instead of the plexiglass tube, a wooden box, 16" x 12" x 10" was used during induction. The cat usually resisted being put in the tube and the ensuing struggle seemed unnecessarily disruptive to the experiment and frightening for the animal. For most of the experiments then the animal was anesthetized in the box, which was also used to transport it from the animal care centre. A high halothane air concentration(4%) was administered directly into the box through a hole in the top.

One drawback to this procedure was that the halothane concentration in the box was not known. Care was taken to remove the cat from the box immediately after induction. This usually took 3-5 mins. during which time the animal struggled and fell about inside the box. This initial reaction was followed by a silent period after which a second movement period occurred. This second period was short in induration and consisted of small movements of limbs and head. As soon as this stopped, the animal was removed from the box and the gas mixture was delivered via the face mask. The concentration of halothane was also reduced (i.e. 50 c.c. hal. air + 900 - 950 c.c. air).

2) Alternate methods tried for peripheral stimulation

In an attempt to increase the incidence of thermoresponsive neurones, alternate methods for peripheral stimulations were tried. In general they were aimed at increasing the surface area and/or intensity of stimulation.

(i) For 2 cats, peripheral thermal stimulation was accomplished by thermal stimulation of the scrotum as well as the face. Facial stimulation was done in the previously described manner. Scrotal stimulation was done by placing a plastic cup filled with hot or cold water over the shaven scrotum for approximately 40 seconds for each thermal test. Stimulations, whether facial or scrotal, were done in random order.

(ii) For 3 cats peripheral thermal stimulation was accomplished by immersion of the animal in hot and cold water. This was done by placing the cat in a modified translucent plastic water container. Hot (45° ct), cold (5° c) or normothenmic (30° c) water was poured directly onto the cat and to a depth so that the animal was in water up to its neck. Between trials for a cell's response to hot and cold stimulation the animal was in 30° c water. This temperature was considered to be neutral as indicated by skin temperature measurements and from the literature (Bligh 1973). The heat pad was placed under the animal, outside the bag.

(iii) One cat was thermally stimulated by surrounding it completely with a plastic bag and then changing the air temperature inside the bag. For this, the animal was shaven so as to reduce the time it would take to induce a change in skin temperature. The same air sources for facial stimulation were used. The air was directed into the bag so as to be deflected around the cat and not directly onto it. Thermometers were placed inside the bag to measure air temperature. Between trials the air temperature in the bag was maintained at a neutral temperature ($\approx$ 30°c) (see #ii above) by short blast from the appropriate air source. A test interval lasted as long as it took to change the air temperature inside the bag from 30°c to 10°c for a cold stimulation and from 30°c to 40°c for a hot stimulation. This usually took a couple of minutes.

3) Central temperature stimulation.

In the final six cats both peripheral and local brain temperature stimulation was done. Peripheral stimulation was facial as described. Hypothalamic temperature was changed by perfusing a pair of 16 gauge thermodes implanted into the brain at an angle 20° anterior to the frame vertical axis such that their tips were located 4 mm on either side of the midline at sterotaxic coordinates frontal +16.5 and vertal -3.0 (Jasper and Ajmone-Marsan, 1954). Hypothalamic temperature was recorded by means of a thermistor probe attached beside and 2 mm distant from a thermode. This distance was the approximate distance the microelectrode was away from the thermode and was therefore a close index of the temperature at the electrode tip.

Stimulation was accomplished by perfusing the thermodes with cold(25°c) and then warm (45°c) water. Between trials, the brain temperature was returned to pre-test temperature (37.0°c) .

Controls for correct microelectrode placement.

(i) An histological control was used to confirm the correct placement of the microelectrode, especially in those experiments where thermo-responsive neurones were found.
Stereotaxic studies necessitate controls for a couple of reasons. One being the possible discrepancy between the exact location of brain structures of the animal being used and those described in the atlas. Slight variations can occur. Another is the possibility of human error in calibrating the electrode. The procedure of calibration can be confusing and has resulted in incorrect placement of the electrode. Therefore some brains were treated and sectioned as described below. The exact location of the electrode tract could be determined under a low powered microscope.
When the animal died or was killed by i.v. injection of 1NKCL causing cardiac arrest, one carotid artery was cannulated and the other tied off while leaving the animal in the frame. The superior sagittal sinus was cut. One hundred millilitres of normal saline were injected through the cannula to clear out the blood, followed by 100 ml of 10% formalin solution to fix the tissue. Coronal cuts were made stereotaxically in front of and behind the electrode tract to establish a frontal plane for sectioning and the brain was then removed from the skull and stored in formalin for at least 2 weeks. In preparation for sectioning, a block of approximately 10 mm on each side was cut around the electrode tract. Dana Orihel performed the sectioning and staining. The block was prepared for mounting by prolonged immension in ethanol, followed by xylene immersion and finally by setting in a paraffin bath. The hardened paraffin cube containing the block was mounted on a holder for sectioning on a microtome. Sections were at 35 m thickness in the region of the

electrode train and one in five was saved. These sections were mounted on slides and stained using a modified Kluver-Barrera stain for cells and fibers and cresyl violet for cells (Kluver-Barrera, 1953).

(ii) In addition to the above histological control, an in vivo control was used. This technique employed the fortunate coincidence of the location of the optic chiasm which lies immediately below the rostal hypothalamus. The end of an electrode penetration was determined by entry of the microelectrode tip into the chiasm. The occurance could be verified by flashing a light into the cat's eyes causing the evoked increase in background noise due to the activation of optic fibers around the elctrode tip.

RESULTS

Two hundred and forty cells were tested for their response to peripheral stimulation and nineteen (7.9%) responded. Fourteen were cold responsive (increased firing rate with cooling and/or decreased it with warming) and five were warm responsive (increased firing rate with warming and/or decreased it with cooling). Fig. 2 illustrates a thermoresponsive neurone. Twenty cells were tested for their response to local temperature change and seven (35%) responded. Four were cold responsive and three were warm responsive.

Table I summarizes the incidence and type of thermoresponsive neurones found using each means of thermalstimulation. The highest incidence of thermoresponsive neurones occurred using local brain temperature changes as the stimulus. In descending order the rest were: facial (9.5%), scrotal (8.3%, but this was unconfirmed), whole body using the bag (5.1%), whole body (excluding the head) using water immersion (4.3%).

RESULTS

Two cells were found which responded to more than one mode of stimulation. One of these was a warm responsive neurone depressed by facial and scrotal cooling tested separately. Another cell responded to both peripheral and local stimulation. It was classed as a cold responsive neurone as it was depressed by facial warming and was excited by local cooling. Occasionally, a cell would respond in the same direction to facial cooling and warming, indicating a tactile input. Usually for curiosity's sake a short time would be spent determining the receptive field on the face and before looking for new cells. One such cell however, also exhibited sensitivity to local stimulation (cold responsive) but was lost before any drug tests could be done.

Excitations and depressions were seen for all five drugs. (Fig. 3 - 12). Table II gives the drug responses and number of responses of neurones in sensitive and sensitve to peripheral cooling and warming. Overall, depressions were twice as common as excitations and about 50% of cells tested responded to at least one drug. Thermosensitive cells were more like to respond to a drug than nonthermoresponsive cells.

Table III shows how many cold and warm sensitive neurones reponded in what way to each drug.

Table IV gives the data correlating multiple drug responses.

The findings for each drug will be presented separately. As discussed in the introduction, iontophoresis allows us to look for - 1) a correlation with thermoresponsive cells, 2) the predicted correlation with thermoresponsive cells and 3) interrelationships among cells responding to more than one drug. These three concerns will be applied to the results for each drug. The significance of any finding will be discussed in the appropriate section.

TABLE I

INCIDENCE OF THERMORESPONSIVE CELLS USING DIFFERENT MEANS OF IDENTIFICATION.

	# Found	# Tested	%
Air	2	39	5.1
water	1	23	4.3
Scrotal	1*	12	8.3
Facial	16	167	9.5
Local	7	20	35.0

* This cell was also responsive to facial stimulation.

TABLE III

DRUG RESPONSES OF NONTHERMORESPONSIVE AND THERMORESPONSIVE NEURONES.

Responses for each catagory are broken down into excitations (+), depressions (-), no response (o), and excitation followed by depression or visa versa (dual).

	NONTHERMORESPONSIVE				COLD RESPONSIVE				WARM RESPONSIVE			
	+	-	0	DUAL	+	-	0	DUAL	+	-	0	DUAL
NA	8	29	38	1	0	6	4	0	0	1	3	0
5-HT	14	20	49	1	0	6	4	0	0	0	4	0
PGE_1	4	5	69	0	0	0	7	0	0	2	1	0
DA	8	18	48	1	1	7	2	0	1	1	3	0
HA	9	11	60	1	0	3	7	1	0	0	3	0

TABLE III

RESPONSES TO PERIPHERAL COOLING (c) AND WARMING (w) OF THERMORESPONSIVE NEURONES ALSO RESPONSIVE TO NA, 5-HT, P.G.E_1., DA, AND HA.

Responses are broken down into excitations (+), depressions (-), dual responses(+ -), or no response (o).

RESPONSE TO DRUG	THERMORESPONSE	NUMBER	TOTAL
NA			
-	cold responsive c+ wo	2	6
	cold responsive c+ w-	2	
	cold responsive co w-	2	
-	warm responsive co, w+	1	1
5-HT			
-	cold responsive c+ wo	2	6
	cold responsive c+ w-	2	
	cold responsive co w-	2	
P.G.E_1.-	warm responsive co w+	2	2
DA			
-	cold responsive co w-	2	7
	cold responsive c+ w-	3	
	cold responsive c+ wo	2	
+	cold responsive co w-	1	1
-	warm responsive c- wo	1	1
HA			
-	cold responsive c+ w-	1	2
	cold responsive co w-	1	
- +	cold responsive c+ w-	1	1
-	warm responsive c- wo	1	1

TABLE IV

CORRELATION OF DRUG RESPONSES

P values indicate statistically significant correlations as determined by the chi-square test.

	NA sensitive neurones		NA insensitve neurones	
	Responsive	Nonresponsive	Responsive	Nonresponsive
5-HT	25(i)	20	11	34
PGE_1	2	38	7	35
DA	24(ii)	16	11	33
HA	18(iii)	27	7	36

(i) $P < .01$ (ii) $p < .01$ (iii) $p < .02$

	5-HT sensitive neurones		5-HT insensitive neurones	
	Responsive	Nonresponsive	Responsive	Nonresponsive
PGE_1	3	36	8	44
DA	21(iv)	15	14	38
HA	15(v)	25	10	47

(iv) $P < .01$ (v) $P < .05$

	$P.G.E._1$ sensitive neurones		$P.G.E._1$ insensitive neurones	
	Responsive	Nonresponsive	Responsive	Nonresponsive
DA	4	6	29	42
HA	2	9	21	56

	DA sensitive neurones		DA insenstive neurones	
	Responsive	Nonresponsive	Responsive	Nonresponsive
HA	17(vi)	21	6	47

(vi) $P < .01$

NA

From Table II, 45/90 (50%) cells tested responded to NA; of these 7 (15.6%) were thermoresponsive and 38 (84.4%) were nonthermoresponsive. Of the 45 cells insensitive to NA, 7 (15.6%) were thermoresponsive and 38 (84.4%) were nonthermoresponsive. Of the NA sensitive cells thermoresponsive (Table III) all were depressed by NA. Six (85.7%) were cold responsive, 1 (14.3%) was warm responsive. Of the 38 NA sensitive nonthermoresponsive cells, eight (21.1%) were excited, twenty-nine (76.3%) were depressed and one (2.6%) showed a dual response. Figures 3 and 4 illustrate a NA depressed and excited neurones respectively.

NA is thermolytic in the cat. If it is involved in thermoregulation one might expect; 1) thermosresponsive neurones to be more likely NA sensitive than nonthermoresponsive neurones 2) warm excited cells to be excited by NA and/or cold excited cells to be depressed. An interrelationship among NA sensitive, 5-HT sensitive and P.G.E. sensitive cells might also be expected since such a finding was previously shown by Jell and Sweatman (1977).

Responses of nonthermosensitive and thermosensitive neurones to NA were alike. On the other hand NA had the predicted effect on most thermosensitive cells; it depressed 6/6 cold responsive neurones but depressed one warm responsive cell (Table III). NA sensitive cells were more likely to be 5-HT sensitive, DA sensitive, and HA sensitive but not P.G.E. sensitive then non-NA-sensitive cells (see Table IV).

5-HT

From Table II it can be seen that forty-one of ninety-eight (41.6%) cells tested responded to 5-HT; of these, six (14.6%) were thermoresponsive and thirty-five (85.4%) were nonthermoresponsive. Fifty-seven cells were insensitive to 5-HT; of these eight (14.0%) were thermoresponsive forty-nine (8.6%) were nonthermoresponsive. All six 5-HT - thrmosensitive cells (Table 111) were cold responsive and depressed. Of the thirty-five 5-HT sensitive, nonthermoresponsive cells, fourteen (40%) were excited, twenty (57.1%) were depressed, and one (2.9%) showed a dual response. Examples of responses to 5-HT are illustrated in Figures 5 and 6.

Do thermosensitive cells respond differently to 5-HT than nonthermosensitive cells? A chi square analysis indicates they do not. Does 5-HT, a thermogenic agent, excite cold respomsive cells and/or depress warm responsive cells as might be expected from the pharmacological evidence? Table III indicates the opposite correlation is found. 5-HT also shows several interrelationships among other drug sensitive cells. From Table IV we see that 5-HT sensitive cells are more likely to be NA, DA, and HA but not P.G.E, sensitive, than non-5-HT-sensitive cells.

P.G.E.

P.G.E. was generally without effect in this study (Table II). Only eleven of eighty-eight (12.5%) cells tested responded; of these two (18.2%) were thermoresponsive and nine (81.8%) were nonthermoresponsive (See Table II). Of the seventy-seven cells insensitive to P.G.E., eight (10.4%) were thermoresponsive and sixty-nine (89.6%) were nonthermoresponsive. Both P.G.E. sensitve, thermoresponsive neurones were warm responsive and depressed. Of the nine P.G.E. sensitive nonthermoresponsive cells, four (44.4%) were excited and five (55.6%) were depressed. Figure 7 illustrates a neurone depressed by $P.G.E._1$.

We expected to find a correlation between cells sensitive to peripheral stimulation and P.G.E. This was not found. However, thermosensitive cells when responsive to P.G.E. were responsive in the way predicted. That is it depressed 2/2 warm responsive cells as might be expected from a thermolytic agent. On the other hand P.G.E. showed as relationship with any of the drug. That is P.G.E. sensitive neurones were not more likely to be N.A., 5-HT, D.A. or H.A. sensitive than non-$P.G.E._1$ sensitive neurones (see Table IV)

DA

From Table II, thirty-six of eighty-nine (40.4%) cells tested responded to DA; of these, nine (25%) were thermoresponsive and twenty-seven (75%) were nonthermoresponsive. Fifty-three cells were insensitive to DA; of these, five (9.4%) were thermoresponsive and forty-eight (90.6%) were nonthermoresponsive. Of the nine DA sensitive, thermoresponsive cells, one (11.1%) was excited (and was cold responsive) and eight were depressed

(seven were cold responsive, one warm responsive). Of the twenty-seven DA sensitive, nonthermoresponsive cells, eight(29.6%) were excited, eighteen (66.7%) were depressed and one (3.7%) was excited then depressed. Figures 8 and 9, illustrate responses to DA.

The responses of thermoresponsive and nonthermoresponsive neurones to DA were not alike. Thermoresponsive neurones not only are more likely to be DA sensitive,(significant at the 5% level), they also respond in the way predicted from central injection studies. Based on these studies (see introduction) in which DA was shown to evoke a fall in cat core temperature, one might expect warm responsive cells (warm excited) to be excited by DA and cold responsive cells to be depressed. Table III indicates that 8/9 thermosensitive cells responded in the predicted way. Further a chi square test indicates that thermosensitive cells are significantly more likely to be depressed by DA than nonthermosensitive cells (significant at the 5% level).

Correlating multiple drup responses in Table IV shows that DA-sensitive cells are more like to be NA, 5-HT, and HA sensitive than non-DA sensitive cells.

HA

From Table II twenty-five of ninety-five (26.3%) cells tested responded to HA; of these, four (16%) were thermoresponsive and twenty-one (84%) were nonthermoresponsive. Seventy cells were insensitive to HA; of these, ten (14.3%) were thermoresponsive and sixty (85.7%) were nonthermoresponsive. Of the four HA sensitive, thermoresponsive cells, three (75%) were depressed (two were cold responsive, one was warm responsive) and one (25%) was both excited and depressed (and was cold responsive). Of twenty-one HA sensitive, nonthermoresponsive cells, nine (42.9%) were excited, eleven (52.4%) were depressed, and one (4.8%) was both excited and depressed. Figures10 and 11 illustrate neurones depressed and excited by HA respectively while Figure 12 shows a neurone having a dual response to HA which is also a thermosensitive neurone.

Like the other drugs a correlation was looked for between thermosensitive cells and HA but was not found. On the other hand, the responses to HA of thermosensitive cells were what one might expect from an agent having a dual temperature effect. That is evidence for both mediation of heat loss and heat gain was found in the depression of two cold responsive cells and the depression of one warm responsive cell. Also, given the possible involvement of H_1 and H_2 receptors mediating different temperature responses it might be expected to find thermoresponsive cells whether cold or warm excited to exhibit a dual response to HA. One such cell was found (see Fig. 12 and Table III.

As for all the drugs except P.G.E., cells responding to HA were also more likely to be NA, 5-HT, and DA but not P.G.E. sensitive than cells not responding to HA (Table IV).

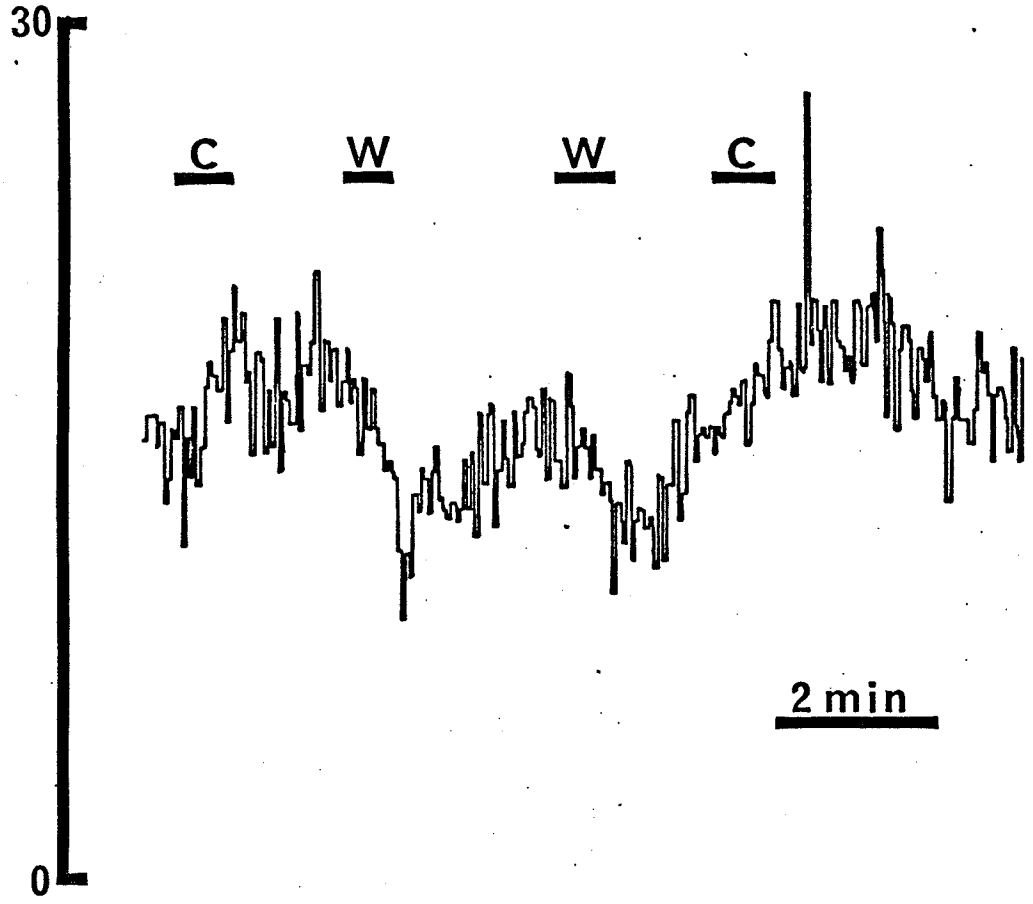


Figure 2. Rate meter record of discharge rate of a cold-responsive neurone. This neurone was excited by facial cooling (c) and depressed by facial warming(w). The ordinate scale is in impulses/second.

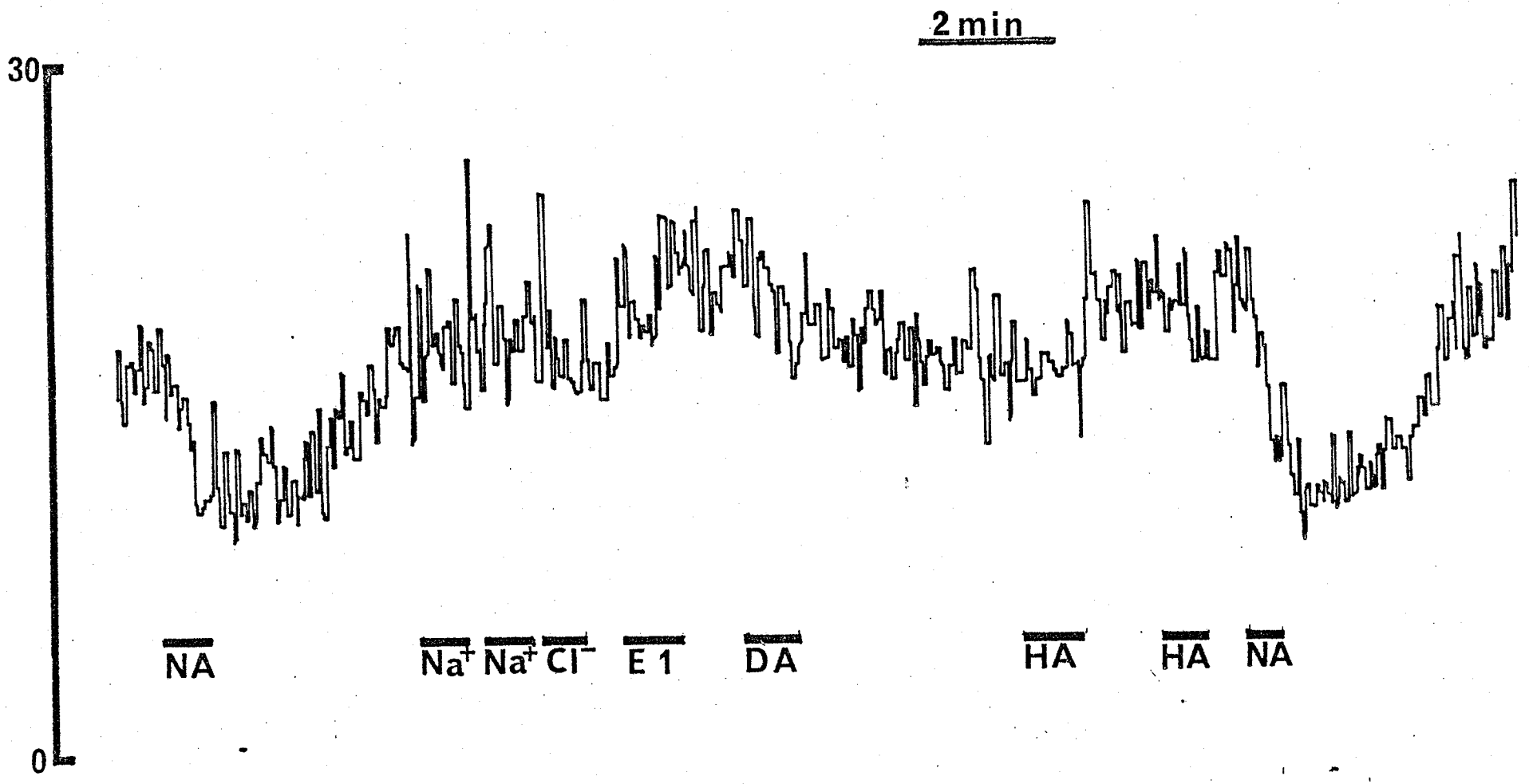


Figure 3. Rate meter record of discharge rate of neurone depressed by two separate applications of NA and unaffected by cation current (Na+). This neurone was also shown to be DA depressed. The apparent E_1 excitation was not repeatable. All cations (Na, 5-HT, DA, HA, Na+) for this and the other figures were ejected for approximately 20 sec. at 50nA and 20 sec. at 100nA. Anions (Cl-, P.G.E.$_1$) were ejected in the same manner at 100nA and 150nA.

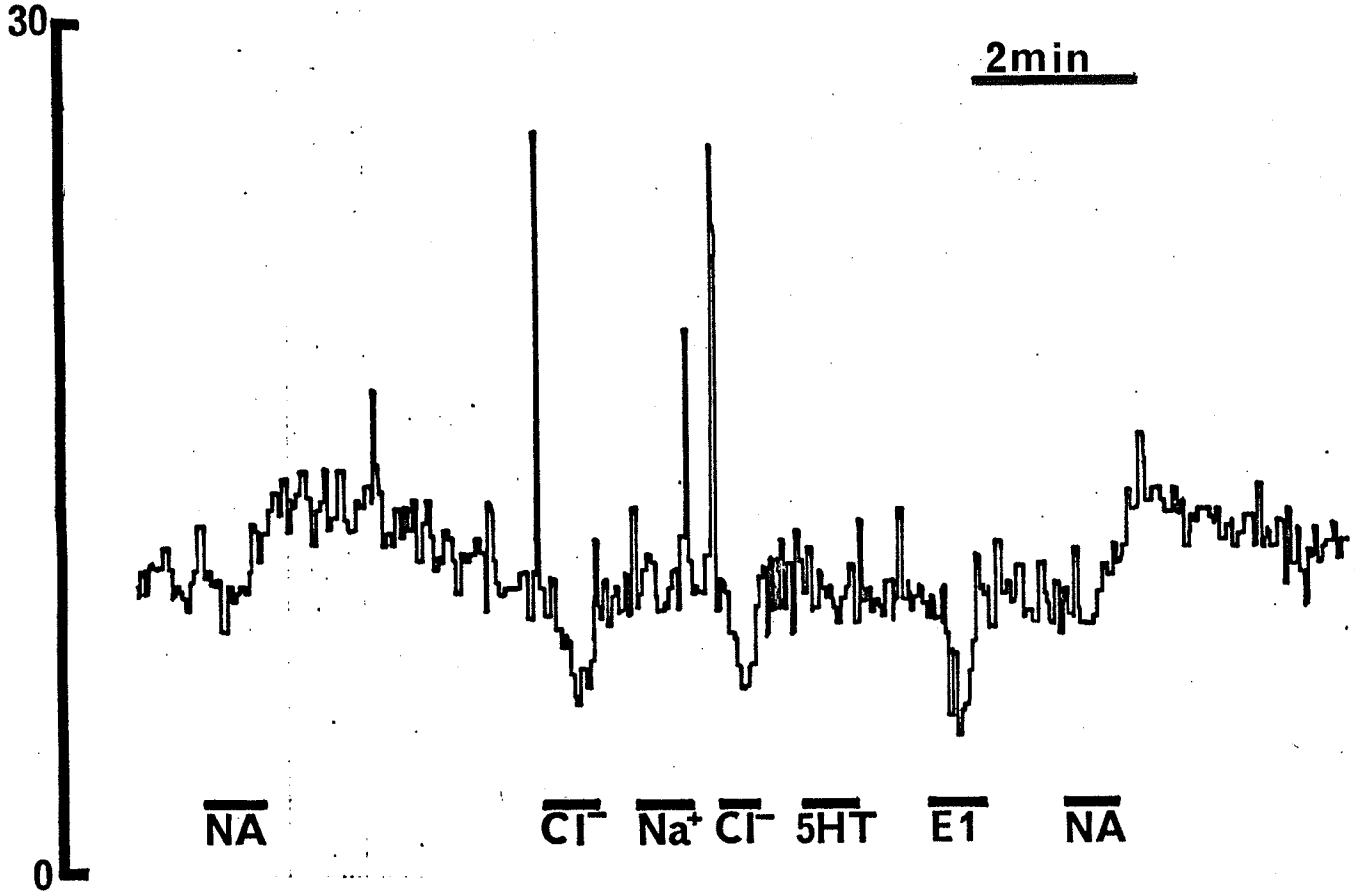


Figure 4. Rate meter record of discharge rate of a neurone excited by two separate applications of NA. This neurone was unaffected by cation current and 5-HT but was anion depressed (ejection parameters as in Figure 3).

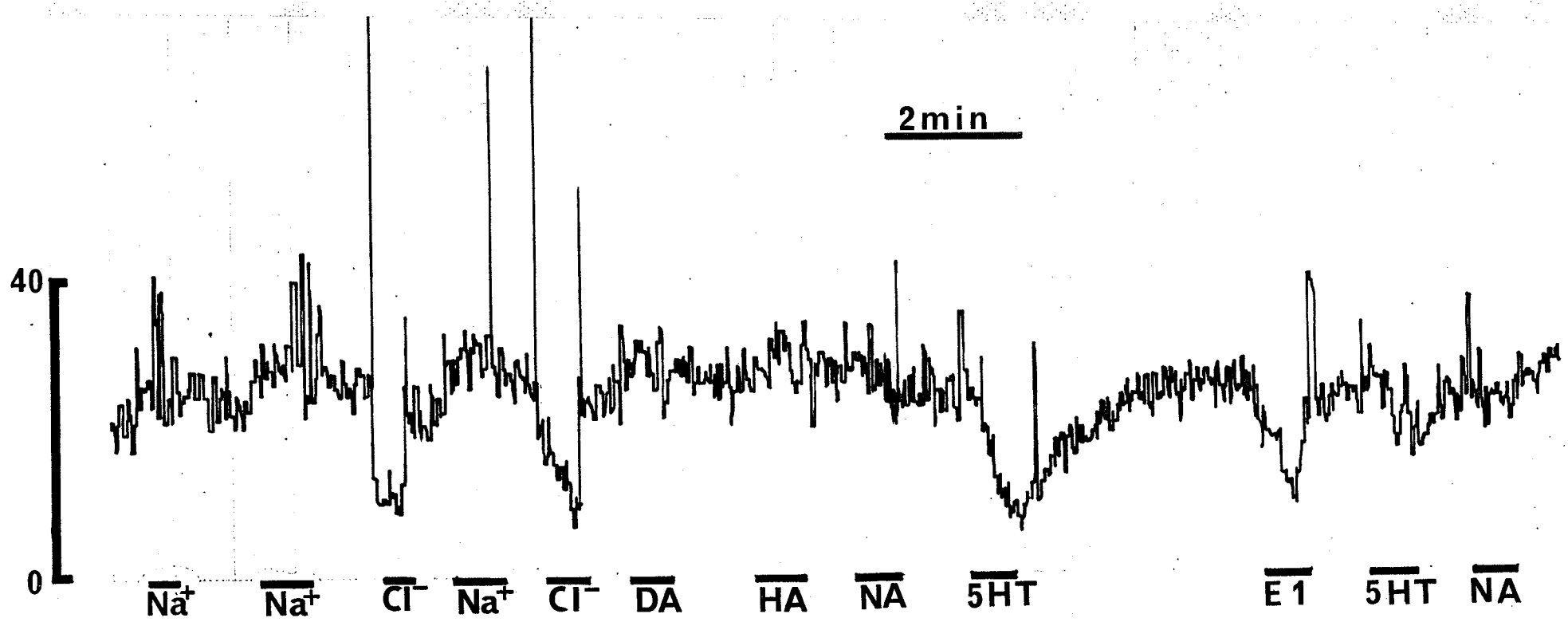


Figure 5. Rate meter record of discharge rate of a neurone depressed by 5-HT and anion current but unaffected by Na+, DA, HA, and NA. The apparent excitations upon the first two application of Nat are artifactual likely due to transient conditions of the recording system. The high lines at the beginning and end of some ejection periods are also artifacts due to the turning on and off of ejection current (ejection parameters as in Figure 3).

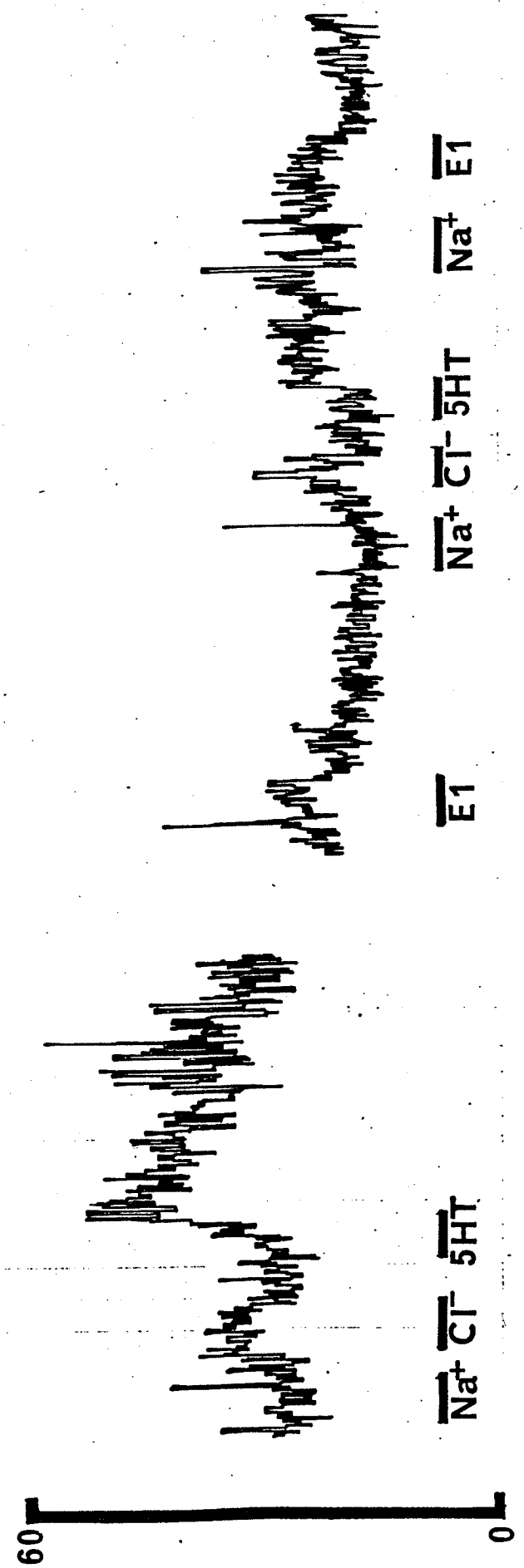
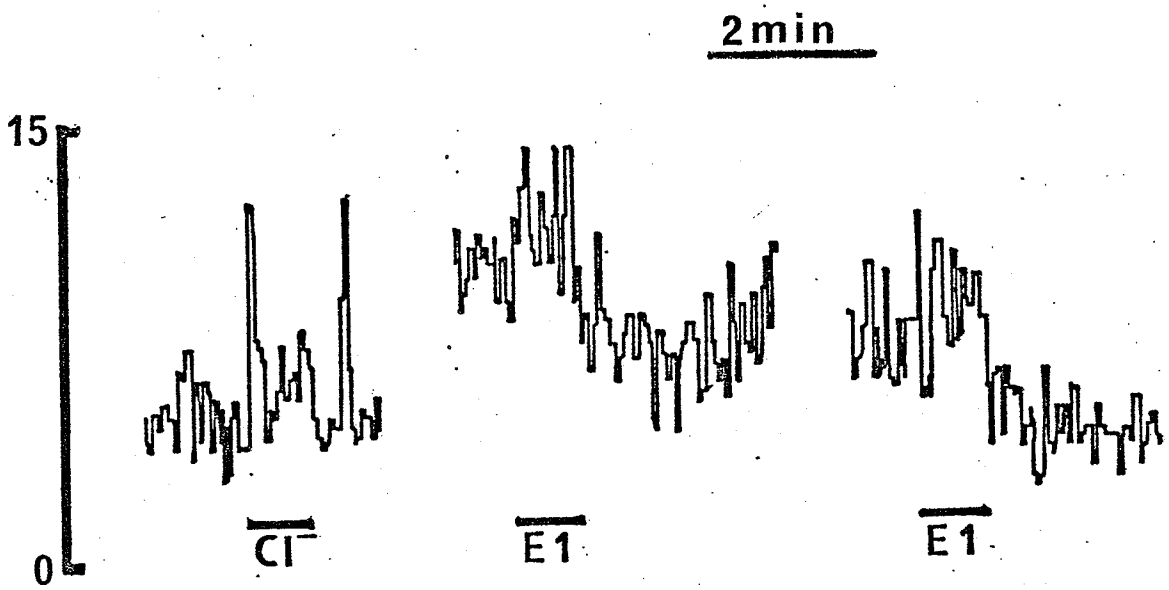
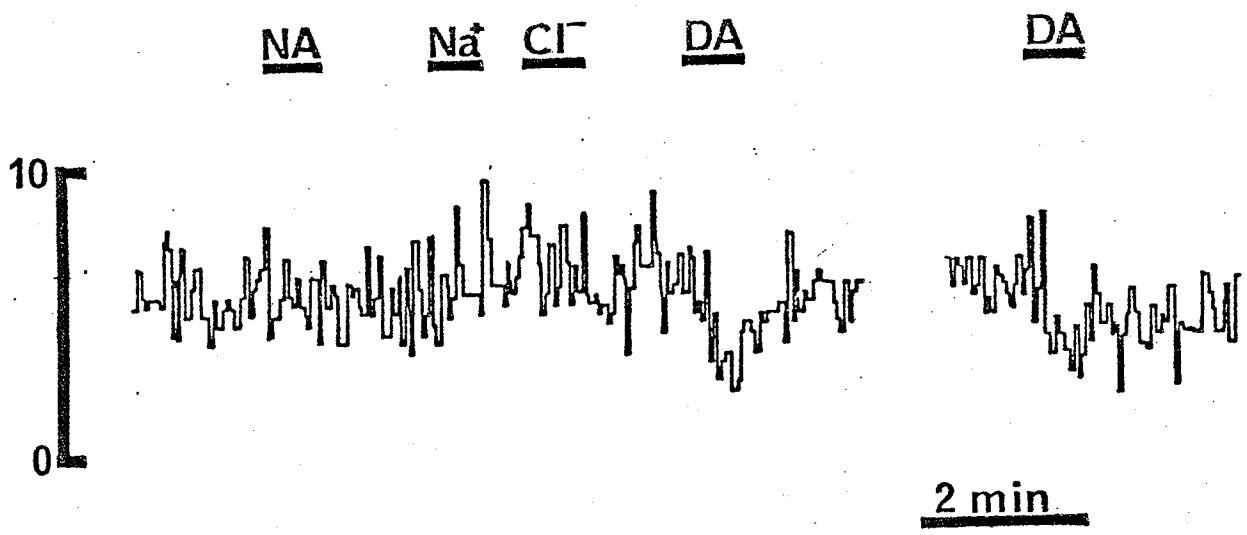


Figure 6. Rate meter record of discharge rate of a neurone excited by 5-Ht and Cl^-. It was also slightly depressed by Na+ and E_1. (See Figure 3 for ejection parameters).



gure 7. Rate meter record of discharge rate of a neurone depressed by two separate applications of E_1 but unaffected by chloride control (see Figure 3 for ejection parameters). Tests were not consecutive thus the discontinuous trace and there was a considerable variation in the 'base line' firing rate.



gure 8. Rate meter record of discharge rate of a neurone depressed by DA and unaffected by NA, Na^+ and Cl^- (ejection parameters as in Figure 3).

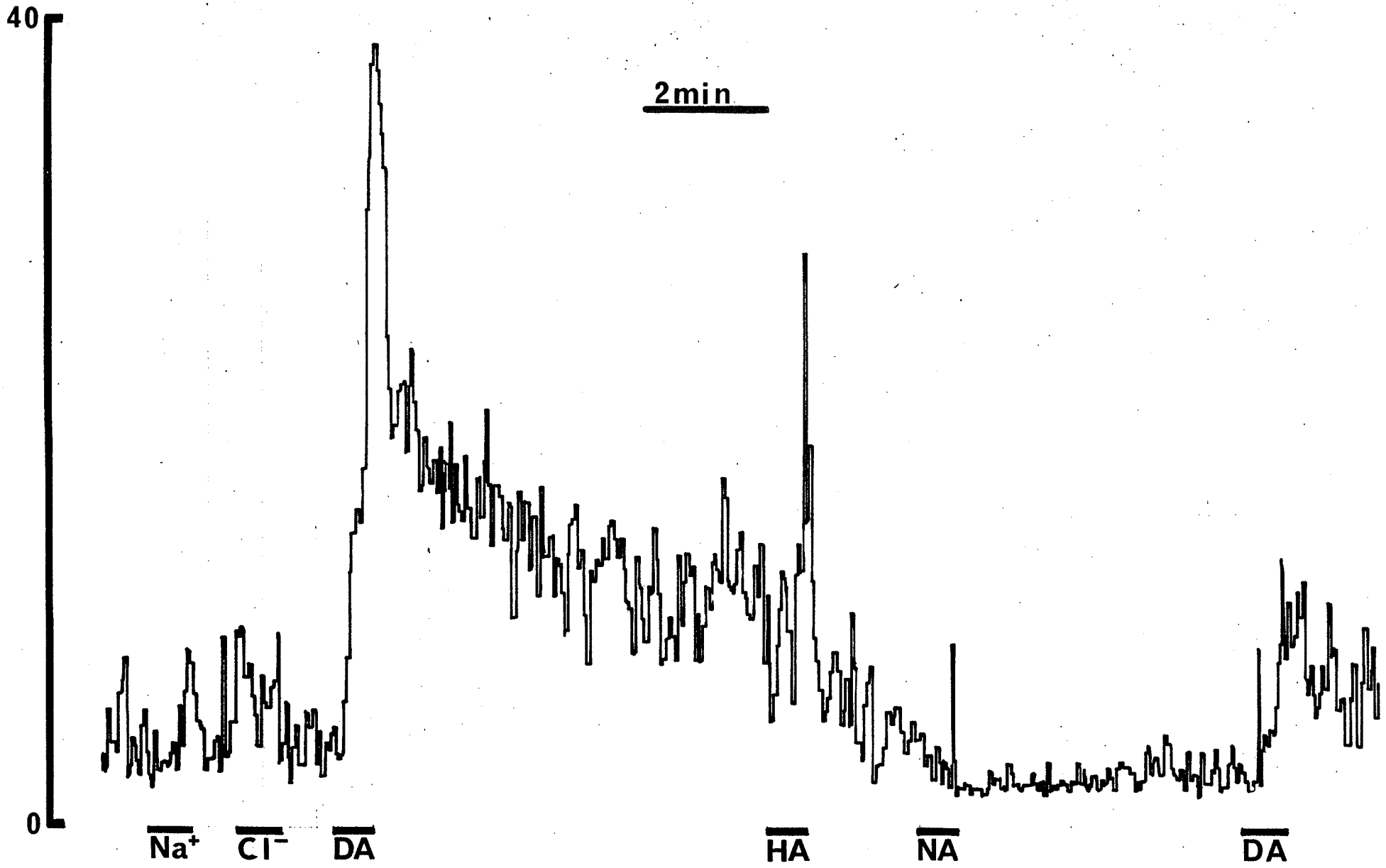
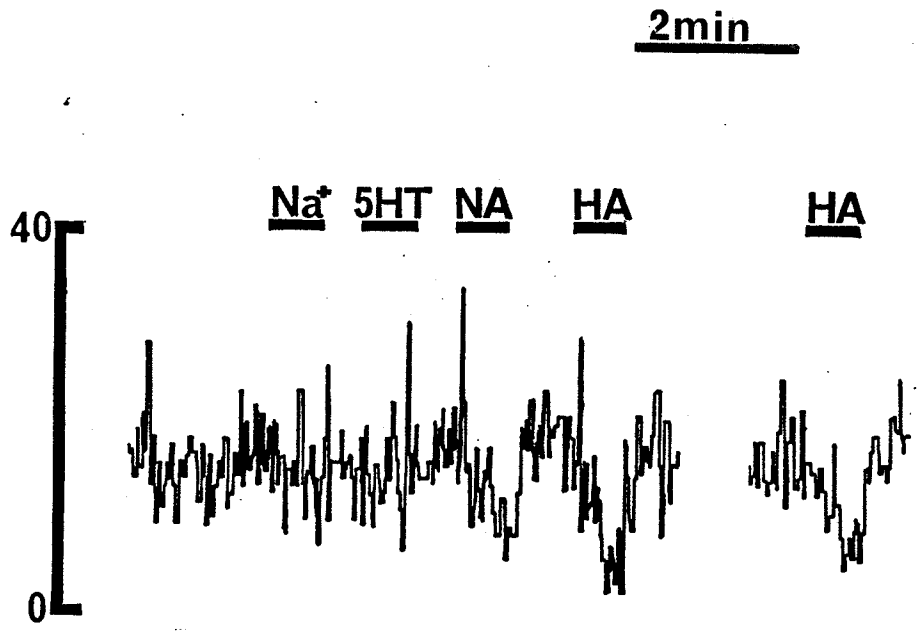


Figure 9. Rate meter record of discharge rate of neurone excited by DA. It was also excited by HA and Cl^- but depressed by NA. Na+ control was without effect. (See Figure 3 for ejection parameters).



gure 10 Rate meter record of discharge rate of a neurone depressed by HA. It was also shown to be NA depressed but unaffected by Na+ or 5-HT (See Figure 3 for ejection parameters).

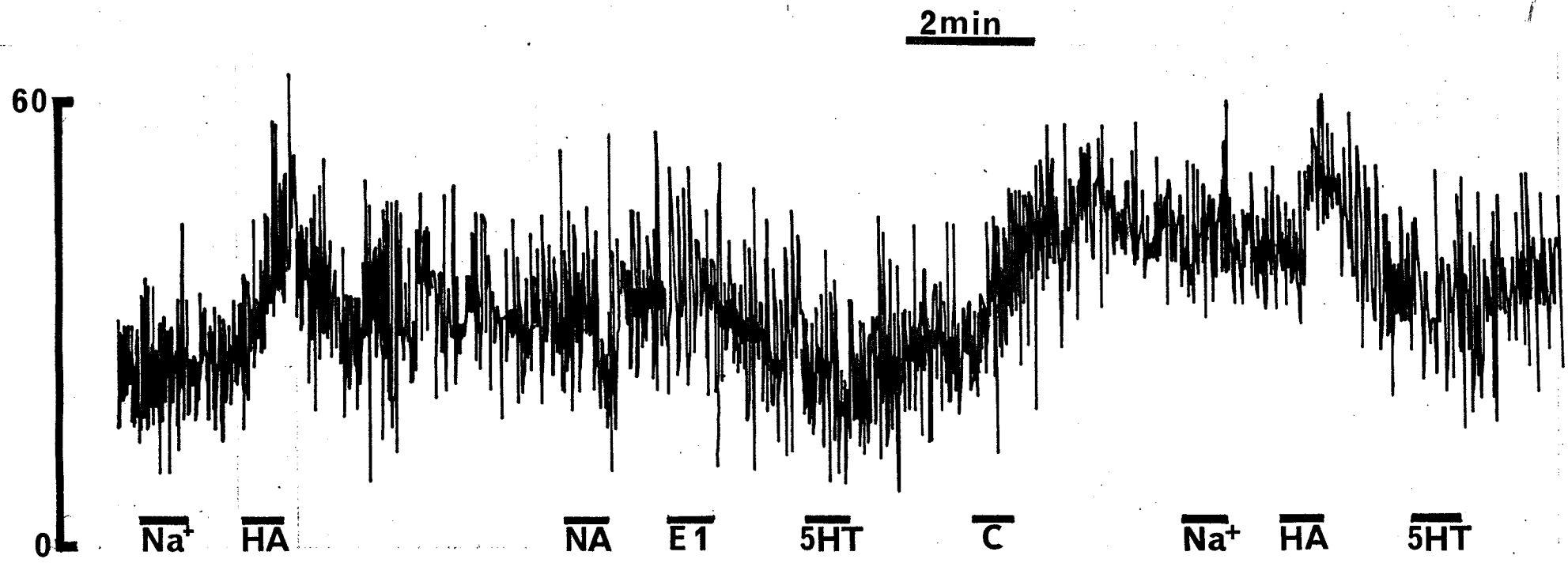
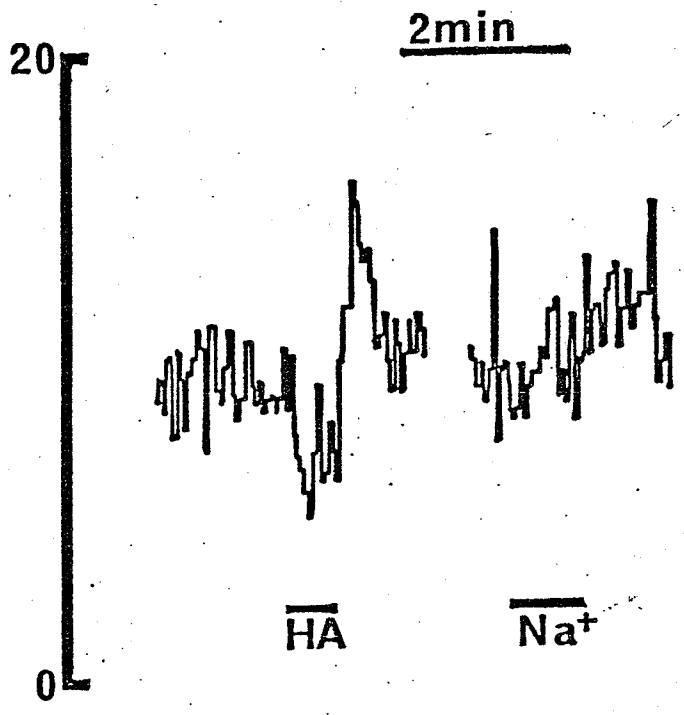


Figure 11. Rate meter record of discharge rate of neurone excited by HA unaffected by Na^+ and E_1 and depressed by NA and 5-HT. The apparent excitation due to cooling (c) was not repeatable (see Figure 3, for ejection parameters).



gure 12. Rate meter record of discharge rate of a neurone depressed then excited by HA but unaffected by current the control. HA and Na+ was ejected for approximately 20 sec. at 50 nA and 20 sec. at 100NA. This cell was also cold responsive.

D I S C U S S I O N

The results from the drug study will be discussed first followed by a discussion on the incidence of thermoresponsive cells. Some problems of interpretation of the drug results will also be mentioned.

NA

In agreement with earlier work done in this laboratory (Jell, 1974, Jell and Sweatman, 1977) responses of thermosensitive and nonthermosensitive cells were alike. However, in disagreement with these reports, where no correlation was seen NA generally affected thermosensitive cells in a way predicted from injection studies, i.e. depressive on cold excited cells. However, this apparent agreement is probably not meaningful (see below).

Beckman and Eisenman (1970), on the other hand, found that NA had the opposite effect on thermoresponsive neurones to that expected from injection studies in cats and rats. NA was generally excitatory on cold responsive and depressant on warm responsive neurones. As mentioned this finding was unconfirmed in the cat (Jell 1973,74), and Murakami (1973) could not confirm the findings in the rat. However, in the rabbit Hori and Nakayama (1973) found that almost all cells responded in the way predicted by the temperature effect of centrally injected NA (and 5-HT). However, these results are generally doubted now because they have not been confirmed and are rather too perfect to be accepted alone.

For some unknown reason this study found more cold excited cells and the incidence of NA depressions were also greater than previously reported (Jell 1974, Jell and Sweatman, 1976, 1977). As seen from Table III depression of cold excited cells was not uncommon which suggests a degree of nonspecificity. Further, the chi square test indicated a lack of correlation not only for thermoresponsive cells and NA sensitive cells but also for the way in which each responded (excited or depressed). Therefore, it would appear this study has not found a correlation between thermalresponse pattern and NA sensitivity. The significance of this finding is still to be discussed.

The mainly depressant action of NA seen in this study agrees will with previous reports. Beckman and Eisenman (1970) found an 80% incidence of inhibition of NA sensitive cells in the POAH of the urethane anesthetized cat. Jell(1973), 1974) and Jell and Sweatman (1976, 1977) observed 50% - 70% of cat, rostal hypothalamic cells responding to NA were depressed.

A predominantly depressive action of NA on POAH neurones of urethane anesthetized rats has been shown (Dyall et al., 1974, Hellon, 1975) as well on the halothane anesthetized rat (Whitehead and Rut, 1974). Geller (1976) iontophoresed NA on cultured arcuate neurones taken from the rat hypothalamus and again depression was the main effect.

ICV administered adrenaline in the dog inhibits most cells recorded from the preoptic area (Winingham et al., 1967). Apparently NA also has a predominantly depressive effect on most C.N.S. cells (Krnjévic, 1974).

The finding reported here of a correlation between NA sensitve and 5-HT agrees with earlier work (Jell and Sweatman 1977), although the lack of coincidence of NA and P.G.E. sensitivity reported does not.

5-HT

This study did not reveal a correlation between 5-HT sensitive and temperature sensitive cells. This agrees with the reports of Jell (1973, 1974) and (Jell and Sweatman, 1977) in the cat and Beckman and Eisenman (1970) in the cat and rat. The latter workers found very few responses to 5-HT which does not agree with the result reported here or elsewhere.

A correlation between 5-HT and thermoresponsive cells was reported in the rabbit. Hori and Nakayama (1973) found that 5-HT excited 15/17 warm responsive cells and depressed 6/7 corld responsive cells. These are as predicted since 5-HT is thermolytic in the rabbit (see Hellon, 1975). As mentioned, this paper is not well regarded. This study found 5-HT inbited 6/6 cold responsive neurones and was without effect on warm responsive cells which is the opposite to that expected from intraventicular and hypothalamic injections of 5-HT (see introduction). However, as discussed with NA, (see previous discussion on NA) the data of Table III is probably misleading. It is concluded then that thermosensitive cells do not respond in a predictable way to iontophoresed 5-HT. The significance of this lack of correlation is discussed jointly with NA and HA on page 48 .

Serotonin appears to be just slightly more inhibitory than excitatory on hypothalamic neurones in the cat, rat, rabbit and dog. Beckman and Eisenman (1970) found it to be largely without effect while Jell (1973) found about equal numbers of excited and depressed neurones. A predominant depressive action of 5-HT emerged in later studies by Jell (1974) and Jell and Sweatman (1976-1977) and in this report (57% depressed).

On the rat Murakami (1973) found 5-HT to excite most cells tested while Beckman and Eisenman (1970) found it to be generally without effect. In cultured tuberal hypothalamic neurones of the rat, Geller (1976) found most cells to be depressed by iontophoretically applied 5-HT. In the rabbit (Hori and Nakayama 1973), 5-HT inhibited slightly more cells than it depressed and, as with NA in the dog, 5-HT administed ICV reduced the firing rate of most preoptic neurones.

The results from hypothalamic neurones agree with those from other C.N.S. structures where 5-HT has been iontophoresed. Both excitations and depressions are found with the latter probably slightly more common (Krnjević, 1974).

The coincidence of 5-HT sensitive and the other drugs except P.G.E. is discussed under 'Some Problems of Interpretation'.

E_1

The lack of correlation between P.G.E. responses and thermoresponsive neurones reported here conflicts with the earlier finding of Jell and Sweatman (1977) in which 77% of P.G.E. sensitive cells were thermoresponsive. A possible reason for the discrepancy is that only ten of the nineteen thermosensitive cells encountered here were tested for their sensitivity to E_1. Perhaps had the other nine thermosensitive cells been tested a greater proportion of these would have been E_1 sensitive than the ten tested. This, of course, is only speculation and the real reason cannot be known. Suffice to say the absence of a correlation between P.G.E_1, thermosensitive cells reported here may be a sampling problem and offers only meagre evidence against such a correlation.

Jell and Sweatman (1977) conclude that "P.G.E. hyperthermia is mediated by a direct action of P.G.E. on thermoregulatory neurones in the rostral phyothalamus". The nature of this action is not clear. One might predict P.G.E. hyperthermia to be mediated by P.G.E. excitation of cold responsive neurones and/or depression of warm responsive cells in the rostral hypothalamus. Ford (1974) makes just such a suggestion since he found 7/7 of the responses of thermoresponsive cells to E_1 fitted this prediction (E_1 excited five cold responsive cells and depressed two warm responsive cells Jell and Sweatman (1977) on the other hand show about 50% of their P.G.E_1 effects on temperature sensitive cells fit the expected and 50% do not. However, there were more cold responsive cells excited by P.G.E_1 then any other category of E_1 - thermosensitive cell types. If cold responsive neurones are for some reason more susceptible to the effect of P.G.E_1 (e.g. more cold responsibe than warm responsive, or they are more accessible, etc.) than warm responsive neurones, then the mechanism of action of P.G.E_1 when centrally injected maybe to excite cold responsive neurones. It is of interest to note that the two P.G.E_1 sensitive, temperature sensitive cells were warm responsive cells depressed by E_1. However, the response of two cells is sparce evidence indeed on which to base a proposal for the mechanism of the thermogenic effect of P.G.E.

Stitt and Hardy (1975) could not show a differential sensitivity to P.G.E_1 of rostral hypothalamic units in the rabbit. The incidence of P.G.E_1 sensitive cells (90%) was also similar to that reported here (12%). However, no cells were inhibited by E_1 in the rabbit. Jell and Sweatman(1976-77) found between 10% - 20% of cells in the cat rostral hypothalamus responded to P.G.E. The reason for the two-and-half fold variation is unknown. Ford (1974) using a diencephalic island preparation in the decerebrate cat found 24% of cells tested responded to P.G.E_1. Both excitatory and depressant effects of E_1 on cells in hypothalamus were seen in the cat (Jell and Sweatman 1976 , Ford 1974).

A mainly excitatory effect of P.G.E_1 is seen on brainstem neurones of cats, reticular, cerebral cortex and hippocampal cells of the rat and on spinal cord neurones of the frog(see Coceani review, 1974).

A major disagreement found in this study with earlier work done by Jell and Sweatman (1977) is that P.G.E. sensitive cells were not also likely to be sensitive to 5-HT or NA. In fact, P.G.E. sensitive cells were not likely to be sensitive to any other drug. It is possible the P.G.E. used in this study was of reduced potency although there is no physical evidence indicating reduce potency. However, the extreme difference obtained using apparently identical techniques and procedures tempts one to invoke an unknown technical source to account for the discrepany. There is no other satisfactory explanation.

DA

DA has never before been iontophoresed on thermoresponsive cells in our effort to correlate its effect with temperature responses. As already stated, thermosensitive cells were more likely to respond to and be depressed by DA than nonthermoresponsive neurones. These relationships are highly statistically significant and are in agreement (albeit not perfect) with what might be expected from the pharmacological studies of ventricular and hypothalamic cannulation experiments.

The apparent relationship between DA sensitivity and thermoresponsiveness may offer a partial explanation at the cellular level of how DA causes a fall in body temperature when administered centrally in the cat. Under thermoneutral conditions, ICV injected DA induced a hypothermia in the cat which is associated with an increase in ear temperature, flushing of the nasal skin and reduced motor activity (Kennedy and Burks, 1974) Intra-hypothalamic DA injection induces in the cat a hypothermia (Quock and Gale , 1974) which is associated with reduced motor activity but no apparent change in ear temperature or respiratory rate (Quock, personal communication). It appears, then, that DA may be activating heat loss mechanisms and suppressing heat producing mechanisms when injected ICV, but only suppressing heat producing mechanisms when injected directly into the rostral hypothalamus. The predominantly depressive action of iontophoresed DA on cold responsive neurones could have the effect of reducing heat production by inhibition of the hypothalamic cold responding system. Perhaps a minimal level of activity of this hypothetical system is required to maintain normal body temperature even under non-cold stressed conditions. That these cells are active under non-cold stressed conditions is evidenced by their spontaneous activity at thermoneutrality.

There is evidence for such a role for medial preoptic neurones in the cat. Squires and Jacobson lesioned this region and observed a chronic decrease of 2 - 3°C in mean colonic temperature in cats kept at ambient temperatures between 19 and 27°C. They attributed this hypothermia to the destruction of cold sensitive structures which are concentrated in this region and which are active under thermoneutral conditions. Our observation of DA depression of cold responsive cells then could explain the hypothermia observed Quock and Gale when DA was microinjected into the cat preoptic anterior hypothalamus as well as the apparent decreased heat production observed by Kennedy and Burks after ICV injection of DA in the cat.

In general our results showing DA depression of hypothalamic neurones agree with those reported by others. Dyball et al found eighteen DA depressions in nineteen DA responsive preoptic neurones in the urethane anesthetized rat. In the halothane anesthetized rat Whiehead et al found twenty-seven DA depressed neurones and no excitations of thirty-nine tested preoptic neurones. In the urethane anesthetized rabbit , DA depressed twenty-one of thirty-six responsive periventricular neurones. In cultured tuberal hypothalamic neurones from the rat, DA depressed fourteen and excited only one of nineteen cells tested .

Evidence from other C.N.S. sites where DA has been iontophoresed also support a predominantly inhibitory effect of DA. (See Krnjévic, 1975).

The coincidence of DA sensitivity with NA, 5-HT and HA sensitivity is discussed in "Some Problems of Interpretation".

HA

Thermosensitive cells do not respond to HA in a significantly different way than do nonthermoresponsive cells. The significance of this is discussed in a separate section (page 49).

HA causes a fall in core temperature at low doses and a rise at high doses when injected into a lateral ventricle (Clark and Cumby , 1976). The responses to HA of thermosensitive cells as shown in Table III, offer neuronal evidence of how this could occur. Depression of cold responsive neurones could result in hypothermia through the presumed inhibition of heat production mechanisms and concominant activation of heat loss mechanisms. Conversely, excitation of cold responsive cells or depression of warm responsive cells is a reasonable and accepted notion whereby a rise in body temperature could be evoked. All three effects, depression and excitation of cold responsive cells and depression of warm responsive cells, are exhibited by HA. Two are exhibited by one cell, a cold responsive cell that is first depressed, then excited by HA. The possibility exists that Clark and Cumby's (1976) demonstration in the cat of a hypothemia at low ICV doses of HA followed by a hyperthermia at higher doses is mediated by cold responsive neurones capable of a dual response to HA.

Our observation of dual responses to HA is in keeping with the literature (Haas et al, 1975, Phillis et al, 1968). Responses to HA with both an excitation and a depression appear to be related to the magnitude of the ejection current and therefore presumably dose (Phillis et al, 1968). The demonstration by Clark and Cumby (1976) of a dose-dependent temperature reversal response in the cat could be explained by these dual responses seen in iontophoretic studies. The response reversal to HA of the cold excited cell may have been HA dose dependant (Figure 12).

Our HA results do not agree with those of Haas et al (1975) who found about 80% of HA responsive hypothalamic neurones to be excited by HA in sodium pentobarbitone cats, and in rats (Haas, 1974) anesthetized with the same and/or urethane. We found only 36% were excited. Renaud (1976) recently reported a predominantly depressant action of HA on hypothalamic cells of the rat and other workers have found that HA is mainly depressant in other regions than hypothalamus (see review by Calcutt, 1976).

See page 49 under "Some Problems of Interpretation" for a discussion on the significance of coincidence of HA sensitive cells with other drug sensitive cells.

SOME PROBLEMS OF INTERPRETATION

Because of the nature of microelectrode recording and iontophoresis, there are inherent problems of interpretation of results obtained. As a means of sampling neuronal responses, microelectrode recording is unsatisfactory as it preferentially selects larger cells and smaller cells may be lost in the background noise of the recording system. Our method of recording from and iontophoresing drugs only when spontaneously discharging cells are encountered also creates a sampling problem. If, for example, glutamate driven cells previous silent, were also tested for their response to temperature and drugs, a greater number and probably less biased sample of a cell population might be studied. Furthermore, one can never be sure the response being recorded is a presynaptic or postsynaptic effect. It is possible that a so called drug responsive cell is not sensitive to the drug being ejected but instead some other adjacent cell is responsive and synapses on the cell being recorded. Or a previously silent cell is excited leading to the interpretation that the original cell is excited whereas it may be completely insensitive. These considerations are particularily important when comparing what is thought to be a single cell's response to numerous stimuli.

The general lack of correlation between thermal response pattern and drug sensitivity seen is difficult to explain and interpret. (P.G.E. is discussed earlier) A possible factor impinging on this situation is that the mediatory effects of the putative thermoregulatory agents may exert themselves at sensitive sites away from the neuronal soma (Jell, 1974). Since our recording technique probably involves the soma more than the cell processes, we may not be applying drugs on to the cell surfaces where receptors are most numerous. Hellon (1975) proposes for NA and 5-HT at least the lack of correlation could be explained if an agent has an input role rather than an integrative role in the hypothalamus. As discussed in the Introduction, the anatomical evidence is supportive for such a role for NA and 5-HT. The notion is that NA is released into the hypothalamus upon locus coeruleus activation as is 5-HT upon raphe stimulation. The work of Eisenman (1974) suggests the involvement of these inputs in thermoregulation in that the firing rates of thermosensitive cells were affected by stimulation of these brainstem nuclei.

What of HA? The main piece of anatomical evidence indicating HA involvement in hypothalamic function is that hypothalamus contains the highest level of HA of any C.N.S. structure; some of it contained in neurones and some in mast cells and possibly some contained in glial cells (Schwartz, 1975). Perhaps this unique nature of HA compartmentation in the C.N.S. accounts for the lack of correlation between thermal response pattern and drug sensitivity of hypothalamic cells. Neuronal release of HA may be less important in a thermoregulatory sense than mast cell, glial or some hither to unknown source and it may be that mast cells or glial cells, etc. mediate the thermoregulatory effects of HA. If this is true one would be surprised indeed to find a correlation. However, this is mere speculation. HA receptors have been rather convincingly demonstrated on hypothalamic cells (Schwartz, 1975) and H_1 and H_2 receptors apparently mediate the dual temperature response of ICV injected HA. Furthermore, neurones sensitive to HA have been reported here and one in particular (Fig. 12) responds in a way fitting the notion of a neuronal mediation of the dual response seen in cats (Clark and Cumby, 1974). Perhaps, this positive finding is worth more emphasis then the negative findings.

Another area requiring some comment on interpretation is the finding that thermoresponsive cells are more likely to be responsive to drugs than non-thermoresponsive cells (Table III). This would seem to indicate that thermosensitive cells have a greater synaptic component then nonthermosensitive cells. It could also be interpretated as supportive evidence for the involvement of the drugs used in thermoregulation. Perhaps, the synaptic component of any one agent with thermoregulatory neurones is not great enough to be revealed by a direct correlation between thermal response pattern and drug sensitivity but instead thermoregulatory function is a composited of the synaptic influence from numerous cell types (noradrenergic, sertonergic, etc.).

What does it indicate that, when a cell responded to one drug, it also tended to respond to the other drugs (except P.G.E_1)? That a cell can respond to more than one drug could mean it has more than one receptor type or a receptor type sensitive to more than one agent. Similar findings by Jell and Sweatman (1977) were explained by postulating the particular agents acted on common target neurones. It would appear then that these drugs, or drugs similar in structure may be acting in the same functional system through the same neuronal system.

INCIDENCE OF THERMORESPONSIVE CELLS.

There was about a two fold difference in the incidence of thermoresponsive cells (Table I) using the four different means of peripheral stimulation. However, the sample sizes varied greatly (12- 167) making comparisons difficult. Nevertheless attempts at stimulating the whole body (bag technique) and everything below the neck (immersion) were no more successful at increasing the incidence of thermosensitive cells found in the hypothalamus than facial stimulation. This would tend to indicate either the intensities of stimulation were unequal (a possibility on which little can be said) or that the density of thermal receptors in the facial area is of such a greater magnitude than the rest of the periphery as to account for the difference in incidence of responsive cells. Also, our results might indicate considerable processing of afferent thermal information which might take place before reaching the hypothalamic cells from which we recorded.

The author is not aware of any reports on the relative densities of temperature receptors in the facial versus nonfacial areas of the cat. The proportion of cold receptors versus warm receptors may differ from peripheral area to area and depending on the stimulation technique more of one type may be stimulated. This, in fact, could account for our findings.

There is evidence that warm receptors, in the cat's nose at least (Hensel, et al., 1974), are in deeper layers of the skin than cold receptors. Perhaps, in other skin surfaces there is a differential distribution of thermal receptors. If this is so, then we may be even less able to make meaningful statements about our findings on the incidence of the centrally located thermosensitive cells. For example, if warm receptors in the nose are deeper they are less likely to be stimulated then cold receptors using the blown air technique. On the other hand, while deeper in the skin perhaps there are more warm receptors then cold in the hairy skin around the nose. Who's to predict which type of receptor would be preferentially stimulated?

The notion of convergence also impinges on the interpretation of our results. One hypothalamic cell was depressed by facial and scrotal cooling although the scrotal testing was unconfirmed (i.e. not repeated). Although meagre, this is evidence of convergence of afferent peripheral thermal input to the hypothalamus. Only the twelve cells tested for response to scrotal stimulation were tested for response to more than one temperature stimulation technique. That this cell was warm responsive agree with findings in the rat by Jahns (1975) whose results would indicate more warm receptors then cold are associated with the scrotum. It appears possible then that some of the hypothalamic cells we recorded from receive inputs from peripheral temperature sensors after convergence of this information.

What is the role of these thermoresponsive neurones? Do they have a function in the homeostatic control of body temperature? That cells responding to changes in peripheral skin temperature are found in the rostral hypothalamus (Wit and Wang, 1968, Hellon, 1970, Jell, 1973) a major site for the autonomic control of body temperature, would indicate intuitively that indeed they are thermoregulatory neurones of some sort. Cells in the thalamus (Jahns, 1975) and the cerebral cortex (Hellon et al, 1973) have been found which also respond to changes in skin temperature. A ready explanation of the existance of these cells is that they are involved in the conscious perception of the thermal state of the animal. But the hypothalamus is not generaly considered a site mediating conscious perceptions. What is the evidence then that these rostral hypothalamic neurones which respond to peripheral temperature stimulation are involved in autonomic thermoregulation?

That changes in skin temperature alone can evoke autonomic thermoregulatory responses is well established (see Bligh, 1973) but the involvement of the hypothalamus and specifically the neurones discussed is unconfirmed by such studies except perhaps by the work of Wit and Wang in 1968. They recorded extracellularily from rostral hypothalamic neurones in the cat while subjecting the animal to a heat stress and monitoring hypothalamic temperature. A small percentage of the cells recorded increased their activity after 2-6 min. of heat stress before hypothalamic temperature rose. Presumably these cells were being driven by skin temperature receptors although it is very possible that deep body

sensors were being activated. When the heat stress was left on long enough to allow hypothalamic temperature to rise, most of these cells showed a further increase in firing rate and panting occurred. Hellon (1970) showed similar findings in rabbits where he found 6 of 8 cells responding to peripheral stimulation also responded to local temperature. He did not, however, mention any thermoregulatory responses. One cell in our study was responsive to both local temperature change and facial stimulation. In fact it was excited by local cooling and depressed by facial warming. Assuming hypothalamic temperature to be a controlled variable, and the existance of hypothalamic temperature sensors, then it appears that convergence of peripheral receptor information and central receptor information occurs at or before the rostral hypothalamic cells responding to peripheral thermal stimulation. Because changes in hypothalamic temperature can evoke thermoregulatory responses and that the cells responding to changes in hypothalamic temperature are probably central thermoreceptors and therefore directly involved in the thermoregulatory pathway, it is reasonable to assume that at least some of the cells responding to peripheral thermal stimula are also involved in the thermoregulatory pathway.

To summarize then, direct evidence in spport of involvement in thermoregulation of rostral hypothalamic neurones responding to peripheral temperature stimulation is scarce. Nevertheless, it seems reasonable to assume they are involved because of two findings: 1) they are situated in a part of the brain mediating some homeostatic thermoregulatory responses 2) some of these cells are also responsive to local temperature change and therefore appear to be receiving information from what are not unreasonably presumed to be central temperature receptors.

We found a predominance of cold responsive neurones (14/19). Jell (1974) found 16/27 temperature sensitive cells were of the cold responsive type. Hellon (1970) had similar results in the rabbit. These results from peripheral stimulation studies are opposite to those from local stimulation studies where most of the cells found are warm responsive (see Bligh, 1973 , and Hensel review, 1974). A factor which could be influencing this apparent opposite situation is inadequate facial warming. As mentioned, warm receptors, in the cat's nose, at least, are in deeper layers of the skin (Hensel et al, 1974) and might therefore be less readily stimulated.

Perhaps our means of facial stimulation and Hellon's whole body stimulation is such that cold receptors are preferentially stimulated. However, this is speculation and too little is known of what constitutes a physiological thermal stimulation and of what is the degree of convergence of thermal information to warrant any further meaningful discussion on this situation.

SUMMARY AND CONCLUSIONS

This study has sought to identify rostral hypothalamic cells in the methoxyfluane anesthetized cat by their response to peripheral thermal stimulation. Cells responding to such thermal stimulation are presumed to be thermoregulatory neurones. Cells were further characterized by their response to iontophoretically applied drugs thought to be involved in thermoregulation. Using these approaches it was hoped that evidence, at the neuronal level, could be obtained for or against the involvement in thermoregulation of noradrenaline, 5-hydroxytryptamine, prostaglandin E_1, dopamine, and histamine.

Dopamine and histamine have never before been iontophoresed on neurones identified by their response to temperature and therefore the results reported for these drugs are original. A statistically significant relationship between thermosensitive cells and dopamine sensitivity was found which suggests the hypothermia evoked upon central injection of dopamine is mediated by dopamine depression of cold excited cells. While no statistically significant relationship with histamine and thermoregulatory neurones could be demonstrated, evidence was obtained which can be used as a partial explanation at the neuronal level of the observed dual effect on temperature upon introcerebro-ventricular injection of histamine in the cat. The results for noradrenaline and 5-hydroxytryptamine generally agreed with previous reports but the results for P.G.E_1 did not. The results are discussed in terms of previous work reported in the literature and in terms of the proposed neuronal pathways mediating thermoregulation.

While primarily a pharmacological study, this thesis also includes a study comparing the incidence of thermosensitive neurons using different means of thermal stimulation including local brain temperature stimulation. The main finding was that various methods of 'whole' body stimulation did not yield a higher incidence of thermoresponsive neurones than facial thermal stimulation. The significance of this is discussed in terms of peripheral receptor distribution and information processing and convergence.

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