AN INVESTIGATION OF THE NATURE OF PUTATIVE SYNAPTIC TRANSMITTERS IN THE RAT CORPUS STRIATUM

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ABSTRACT

The role of the biogenic amines dopamine and acetylcholine, and the amino acids glutamic, aspartic and gama-amino-butyric (GABA) acids in the rat corpus striatum has been investigated by using microiontophoretic application of these and related substances to striatal cells in conjunction with stimulation of the corticostriate afferent pathway.

In keeping with the findings of earlier workers, application of dopamine (DA) and acetylcholine (Ach) to striatal neurones resulted in both excitatory and depressant effects. The frequency of encountering excitatory effects was markedly lowered by the use of barbiturate anaesthesia (Dial CIBA), especially excitations produced by Ach. For this reason a penthrane - air anaesthesia system was developed and used in this study.

Cortical stimulation generally resulted in depression of glutamate induced striatal neurone firing, usually following either a brief excitation (28 ms) or after an equally brief latency (30 ms). Striatal units that were initially inhibited by cortical stimulation had a higher probability of being depressed by catecholamines, otherwise there did not appear to be any relationship between the response to stimulation and the responses to Ach, DA and noradrenaline (NA).

D-amphetamine, iontophoretically applied, was found to depress almost all striatal cells to which it was applied. Apomorphine (APO), iontophoretically applied, exhibited dopamine agonist actions. Spiroperidol, a butyrophenone which antagonises the behavioural effects of DA and APO, when administered systemically (i.p.), antagonised the depressant actions of Ach, DA, NA and APO on striatal cells and markedly increased the number of cells excited by DA and NA but not by APO or Ach. This effect did not appear to be mediated through a direct effect on dopamine receptors.

Bicuculline methyl iodide ejected at current levels sufficient to block GABA induced depression of striatal cell firing, caused a marked increase in cellular excitability and enhanced the initial excitation resulting from cortical stimulation, suggesting the presence of an intrinsic GABA mediated inhibition of striatal neurones.

Glutamic acid di-ethyl-ester (GDEE), reported to be a glutamate antagonist, reversibly blocked striatal cell firing ($\bar{x}$ = 12ms latency), in response to cortical stimulation, as well as excitatory amino acid induced excitation of the same cells with little alteration in spontaneous firing. This suggests very strongly that the excitatory amino acids; glutamic or aspartic, function as the transmitter in the excitatory fine-fibre cortico-striatal projection.

GENERAL INTRODUCTION

The corpus striatum, which is taken to include the caudate nucleus and putamen, remains one of the more enigmatic regions of the mammalian brain, despite the large amount of research effort that has been focussed upon its neuroanatomy, physiology and pharmacology. Virtually all of the ideas concerning its physiological function remain largely speculative.

The striatum accounts for approximately 10% of the total brain volume in mammals and increases in anatomical complexity as one ascends the phyllogenic scale. In rodents the striatum is a single entity, the division into a separate caudate and putamen by the coalescing internal capsule fibres becomming more marked in the higher mammals.

Neuroanatomically the striatum is remarkably unstructured. Golgi studies have shown that it consists largely of small interneurones which have relatively extensive globular dendritic fields and short axonal processes[15]. The cells comprise in excess of 90% of the total neuronal population. Other cell types account for a further 7 - 8% and only 2 - 3% of the striatal neurones; large diameter spindle shaped cells, have axons which leave the striatum. These efferent cells do not appear to have any particularly obvious relationship with the remaining striatal interneurones, and appear to be scattered more or less randomly throughout the striatum. Fine poorly myelinated or unmyelinated axons, which appear to be striatal afferents, form a network of fibres which are intersected by the dendritic trees of the interneurones in a manner somewhat analagous to the cerebellar granule cell fibres.[16,17].

This apparently unstructured aspect of the striatum is one of the root causes for the difficulties encountered in divining its function and physiology

since such an amorphous structure is not readily amenable to the same e experimental manipulations as are the cerebral cortex, hippocampus or cerebellum.

The striatal outflow appears to project to only two regions of the brain, the globus pallidus and the pars-reticularis of the substantia nigra [29]. A striato- cortical projection has been suggested by many workers but no compelling evidence for the existance of such a pathway has yet emerged. In fact the striato - pallidal projection appears to consist of collaterals of the striato-nigral fibres[28]. No direct projection either from the striatum, pallidum or S.N. to motor nuclei has been described, although this system continues to be referred to as the extra-pyramidal motor system.

In contrast to the restricted outflow, the striatum receives a wide convergence of inputs. Afferent fibres derive from the entire ipsilateral cortex and from the sensori-motor area of the contralateral side, and are somatopically distributed within the nucleus,[3,4,26,27]. as are the fibres from intralaminar thalamic nuclei. Fibres also project from the substantia nigra[1,10,30] and a number of smaller mesencephalic nuclei. The diffuse, unmyelinated dopaminergic projection from the substantia nigra pars-compacta have been the focus of a great deal of research following the demonstration of dopamine involvement in the 'extra pyramidal' symptoms of Parkinson's disease and related pathologies. The development of the fluorescent histochemical techniques for visualising catecholamine pathways by Anden et al[1]. has served to intensify this interest. Recently the presence of a parallel larger diameter non-dopaminergic nigro-striatal pathway has also been described [10]. Striatal afferents also derive from the vestibular,[24] auditory and possibly from the raphe nuclei as well.

Because of this wide convergence of input and the very restricted nature of the striatal outflow, the striatum is generally held to perform some sort of integrating function - presumably of 'slow' activity[7]. Striatal units have been described which fire in advance of ramp type voluntary movements in monkeys[8,18]. Divac has suggested on the basis of phyllogeny that the corpora striatum represent vicarious cerebra[9], and that by analogy they may perform functions analogous to the cerebral hemispheres. However the restricted nature of the striatal outflow poses a considerable interpretational problem, since striatal activity per se does not appear to exert a direct effect on motor pathways. Striatal stimulation results in the inhibition of pallidal neurones[22,28] which normally appear to have a relatively high tonic discharge rate. Behaviourally such stimulation results in a 'freezing' reac tion following several seconds delay, but does not result in discrete motor responses[6,32].

Pallidal neurones project in turn to the mid-brain tegmentum; sub-thalmic nucleus and the intralaminar thalamic nuclei among other sites, while the projections from the substantia nigra do not appear to be known with any certainty. From behavioural studies it would appear that there is a caudal projection from the substantia nigra to the tegmental reticular formation and possibly a decending spinal projection, since nigral stimulation has been reported to augment spinal root to root reflexes[31]. Striatal stimulation also gives rise to mossy and climbing fibre responses in the cerebellum which appear to be mediated by the globus pallidus and substantia nigra[11].

The presence of a possible striato-pallidal-thalamic-cortex-striatum pathway has been held to imply the presence of a functional negative feedback loop - the 'caudate loop' ; although the evidence is largely

circumstantial. A similar striatal - nigral - striatal loop involving the dopaminergic nigro-striatal fibres and the GABA containing striato-nigral pathway has also been postulated. The presence of the parallel excitatory nigro - striatal projection complicates matters however[10].

Stimulation of striatal afferent pathways seldom gives rise to action potentials from striatal cells[2,13] yet virtually all cells from which intracellular records have been made respond to cortical and thalamic stimulation with EPSPs followed by IPSPs. The failure of striatal inter-neurones to fire has been presumed to be due to a constitutional peculiarity of the cell membrane or due to the presence of tonic inhibition[19]. For this reason the striatum has long been known as one of the 'silent' areas of the brain. Intra-striatal stimulation also results in a similar EPSP-IPSP sequence to that produced by afferent path stimulation, the latency of the locally induced response indicates that it is polysynaptically mediated[19].

The relatively homogeneous structure of the striatum, and , at least in the cat and rat relatively diffuse distribution of the afferent fibres makes interpretation of local field potentials and their drug induces changes exceedingly difficult.

One technique that holds promise for aiding the understanding of striatal function is that of iontophoresis. Ideally the technique attempts to mimic the effects of post-synaptically released transmitter substances on the cell under observation, the neuronal response generally being inferred from changes in firing rate or pattern, although intracellular recording yeilds far more information. Allowing for the fact that there are associated with the neuronal membrane a large variety of other structures;

glia, astrocytes, parts of other neurones etc., which can greatly affect the access of the applied substances to the target cell; many results int the CNS obtained by using this technique have been remarkably consistent with results obtained from neurochemical and pharmacological studies.

Since the effect, excitation or depression, of an iontophoretically applied substance is generally assessed by its effect on the spontaneous or stimulus induced neuronal discharge, the 'silence' of striatal neurones necessitates the simultaneous application of an excitant compound, traditionally glutamic acid, to raise the firing rate of the cell under investigation sufficiently to observe drug induced changes.

Since the striatum contains among the highest concentrations of dopamine[12] and acetylcholine esterase in the CNS, both dopamine and acetylcholine have been applied iontophoretically to striatal neurones in the hope of determining their possible roles. The results obtained to date have been equivocal, though cells inhibited by nigral stimulation appear to have an increased probility of being depressed by dopamine[5]. The relative importance of acetylcholine in the striatum is inferred from the high levels of acetylcholine esterase and choline acetylase present[20,23] and the behavioural effects of intrastriatal injections of cholinergic agonists such as arecoline and oxytremorine[21] and of antagonists such as atropine, even though the pharmacological specificity of these substances has not been completely established.

The striatal efferent neurones appear to be the source of the acetylcholine esterase activity in the striatum (Deadwyler pers.com.) and produce prodigious amounts of the enzyme, sufficient it appears to account for the high concentrations reported. Yet these same neurones appear to release GABA at their nigral and pallidal terminals[25]

Much of the speculation as to the role of ACh and dopamine in the

striatum has been based on evidence obtained from the behavioural responses to intrastriatally administered drugs following unilateral striatal or nigral lesions. Cholinomimetics tend to produce responses which are reciprocal to those produced by dopamine or dopamine agonists such as apomorphine which tend to cause the animal to rotate towards the side opposite the lesion (contralateral rotation) [21] . Thus the butyrophenone tranquillisers such as haloperidol cause the animal towards the lesioned side, indicating that it in some manner blocks the actions of dopamine. Because of the gross nature of the lesioning and the relative ansence of a defined neuronal architecture it is exceedingly difficult to relate the drug effects observed in these studies to specific functional groups of cells.

Other possible transmitter substances ; glutamic acid and GABA have also been reported to be present in unusually high concentrations in the striatum [14] but have not attracted any attention. Since both these substances have been demonstrated to be putative neurotransmitters in other systems this lack of attention seems remarkable.

In the light of the foregoing, then it was decided to investigate the nature of the synaptic substances which might be involved in the cortico-striatal pathway. Since striatal cells receiving cortical afference might-also recieve a particular pattern of other inputs, particularly nigral and intrastriatal; the pharmacological response pattern of these striatal cells responsive to cortical stimulation was also investigated.

In carrying out these investigation it became necessary to develop a number of new techniques for anaesthesia, iontophoresis of drugs and for data processing. These appear in the appendix at the end of the dissertation.

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ALTERATIONS BY ANAESTHETIC AGENTS OF THE RESPONSES OF RAT STRIATAL NEURONES TO IONTOPHORETICALLY APPLIED AMPHETAMINE, ACETYLCHOLINE, NORADRENALINE AND DOPAMINE.

ABSTRACT

The effect of two anaesthetic agents, penthrane and di-allyl barbiturate (Dial , CIBA) was investigated on the responses of rat striatal neurones to iontophoretically applied noradrenaline (NA), dopamine (DA), acetylcholine (ACh) and d-amphetamine (d-AMPH).

At both high (50mg/Kg) and low (35 mg/Kg) dose levels of Dial there was a highly significant (p 0.1%) change in the nature of the responses to all the substances tested. This change was primarily a decrease in the frequency of excitatory responses observed to catecholamines and to ACh, compared to those observed under penthrane.

While the dosage of Dial also affected the nature of the responses it was to a much lesser degree, d-AMPH responses were not affected and were almost purely inhibitory.

Under both anaesthetics, there was no evident correlation between the responses to d- amphetamine and to the catecholamines.

INTRODUCTION

Amphetamines have been postulated by many workers to exert their behavioural effect through the release of presynaptically stored catecholamines[2,4]. The initial enhanced exploratory behaviour seen in mammals following d-amphetamine treatment has been ascribed to the release of noradrenaline, while the stereotyped behaviour seen at higher dose levels has been postulated to involve striatal dopaminergic pathways[6].

Local iontophoretic application of d- amphetamine (d-AMPH) to rat brain stem neurones[2] causes excitation while in cat caudate it depresses glutamate induced firing[7]. These observed differences can be ascribed to differences in the recording site, species and the nature of the anaesthetic agents used; halothane in the rat and Dial - urethane in the cat. In addition the effects of amphetamine were paralleled by those of noradrenaline (NA)[2] and dopamine (DA)[7], findings which would tend to support the hypothesis that catecholamines mediate the activities of amphetamine.

Since anaesthetic agents have been shown to have profound effects on the pharmacological responses of CNS neurones[3,12,14,19] t was of interest to determine the concommitant effects of anaesthetics on the responses to iontophoretically applied d-AMPH and catecholamines of rat caudate nucleus neurones and to see whether the reported positive correlation between the responses to catecholamines and d-AMPH persists. Such information could help elucidate the mechanism of action of d-AMPH in the caudate nucleus.

METHODS

Hooded rats of both sexes (250 - 500 gm) were used for all experiments. In the initial barbiturate series (15 rats) Dial (di-allyl barbiturate) was given intraperitoneally at a dosage of 50 mg/Kg. In the second series (18 rats) Penthrane (methoxyfluorane - Abbot) was initially given, with air, at a level of 1% for 20 to 40 minutes following halothane induction (1 ml in a closed container). After this period the penthrane level was slowly tapered off to approximately 0.07% and maintained at this level for the remainder of the experiment. At this level the animals were in early stage 3 anaesthesia (corneal reflex still present). Since rats are obligate nose breathers, and since the animals were breathing spontaneously, a rubber snout mask constructed from a gum rubber dropper teat was used to avoid the necessity for tracheal intubation or tracheostomy. The snout mask did not increase the respiratory dead space more than 0.1 ml. The gas mixture was delivered from a simple laboratory constructed anaesthesia machine which permitted the accurate metering and mixing of the fresh and penthrane saturated air streams.

A third series of 3 rats were given Dial at 35mg/Kg following halothane-air induction. This dose gave an anaesthesia level comparable to that acheived under penthrane once the animal was induced with halothane, but was not of itself adequate to induce anaesthesia.

In all series, recording was not commenced for at least one hour following surgery. Haert rate was monitored during the penthrane anaesthesia and would remain between 270 and 320 beats per minute throughout the experiment. The rat, mounted in a stereotaxic frame, lay on a DC operated heating pad[17], a rectal thermistor probe being used to regulate

the animals temperature at 37.4°C.

The skull was opened and a small trephine hole (2 mm in diameter) was made using a stereotaxic drill to allow insertion of the microelectrode in the region of the head of the caudate (A 8.5 mm, L 2.5 mm [11]). Most of the exploratory tracks were confined to the body of the striatum.

A miniature 7 barrel micropipette [18] (tip diameter 5 - 9 microns) was used for extracellular recording and drug application. The centre recording barrel and one or two lateral barrels were filled with 2 M NaCl. These and the drug containing barrels were filled by centrifugation immediately before use.

Drugs used were d-amphetamine sulphate (0.2M, pH 5.6), dopamine HCl (0.2M, pH 4), l-noradrenaline HCl (0.2M, pH 3.5) and in the second and third series of animals, acetylcholine HCl (0.2 M, pH 3.5), partly as a further index of the anaesthetic's effects. Glutamate (as Na glutamate) (0.2M.pH 7) was used to excite ' silent' cells or to increase the firing rate of spontaneously firing cells. Metered iontophoretic injection currents were supplied from a 6 channel FET constant current source[15]. Current controls (+30 to +50 nA passed through a lateral NaCl barrel) were performed on the majority of cells, the responses of any cell which showed significant current sensitivity were disregarded, although current sensitive cells were seldom encountered. Retaining currents used were between -10 to -20 nA.

Neuronal activity was amplified, monitored on an oscilloscope, the Y output of which passed via a window discriminator to an epochal rate-meter[16]. The ratemeter output was plotted with a chart recorder to provide a permanent record of changes in firing rate. The epoch time chosen varied from 0.5 to 3 seconds depending upon the firing rate and firing pattern of the cell under scrutiny.

RESULTS

The most immediately obvious difference between the anaesthetic regimes used was the markedly increased probability (at least 20 fold) of finding, under the light anaesthetic doses, striatal cells which were either spontaneously firing (1 - 5 spikes per second), or which could be excited by low current (2 - 15 nA) applications of glutamate ions. Virtually all cells encountered required some glutamate 'drive' to increase the spontaneous firing rates to a level which permited the recording of drug effects (rates greater than 3 - 5 spikes per second).

This incerased probability of encountering responsive cells under penthrane is not reflected in the ratio of the number of cells tested to the number of animals used (Table 2) because this data was collected as part of another study involving a far more intensive investigation of the responses of each cell encountered.

Quite noticeable differences in the effects of the concentrations of the two anaesthetic agents on the striatal cell firing constancy were evident. Under Dial (50 mg/Kg) cells exhibited a relatively constant discharge rate over prolonged periods (up to one hour), whereas under both penthrane and Dial (35 mg/Kg) many of the cells showed both random and cyclical changes in firing rate, presumably reflecting changing levels of presynaptic drive. Under both anaesthetics striatal cells exhibited two different patterns of firing, both of approximately equal occurrence; irregular firing and bursting; but there appearred to be no particular relationship between firing pattern and the cell's response to applied drugs.

The responses of striatal neurones to the iontophoretic application of d- AMPH, Ach, NA and DA were markedly affected by the anaesthetic agent used. Under Dial virtually no excitatory responses to catecholamines