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COMPARISON OF THE ACTIONS OF OCTOPAMINE AND CATECHOLAMINES ON SINGLE NEURONES OF THE RAT CEREBRAL CORTEX

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1 The technique of microelectrophoresis was used to compare the actions of octopamine, noradrenaline and dopamine on single cortical neurones of the rat.

2 Octopamine both excited and depressed neurones of the cortex. Frequently cells depressed by noradrenaline were excited by octopamine; occasionally the converse was true. The time courses of action of the two amines also differed. Dopamine-elicited excitations were observed, but also were not correlated with octopamine-elicited effects.

3 When octopamine and noradrenaline both caused depressant effects, octopamine frequently was of less apparent potency than noradrenaline. When these amines were excitatory, octopamine appeared at least as and sometimes more potent than noradrenaline.

4 Octopamine was only weakly effective on cortical neurones identified by antidromic stimulation of the pyramidal tract, or synaptically excited by stimulation of the ventrobasal thalamus.

5 α-Flupenthixol and propranolol were without effect on octopamine-elicited changes in firing rate at doses which were effective in blocking the actions of dopamine and noradrenaline respectively. Metoclopramide did not block the actions of any of the three agonists, but had strong effects of its own.

6 The results suggest that receptors sensitive to octopamine, and which appear to be pharmacologically distinct from those previously categorized as noradrenaline and dopamine receptors, may exist on central neurones of the rat.

Introduction

A considerable body of evidence has accumulated which is suggestive of a function for octopamine as a synaptic transmitter in a variety of invertebrate species (Carpenter & Gaubatz, 1974; Kravitz, Evans, Talamo, Wallace & Battelle, 1976; Robertson & Carlson, 1976; Axelrod & Saavedra, 1977; Robertson & Juorio, 1977); however, its role in mammalian nervous tissue has been the source of considerable speculation. It may act as a less potent agonist at noradrenoceptors in the periphery (Lands & Grant, 1952; Fischer, Horst & Kopin, 1965; Kelly & Burks, 1974), and a function at central synapses as a 'synaptic activator' has been proposed (Boulton, 1976).

Octopamine is normally present in many areas of the rat brain including the cerebral cortex (Molinoff & Axelrod, 1972; Saavedra, 1974) and it is depleted from terminals by administration of reserpine (Carlsson & Waldeck, 1963; Chin A Paw, Knegt-Verpalen & Noach, 1968) or 6-hydroxydopamine (Baldessarini, 1971). The high rate of turnover of octopamine (Kakimoto & Armstrong, 1962; Molinoff & Axelrod, 1972) in spite of low tissue concentrations (Buck, Murphy & Molinoff, 1977; Danielson, Boulton & Robertson, 1977) may indicate a more important mechanism of action for this amine in brain than has heretofore been thought likely.

In view of the several proposed roles for octopamine (Hicks, 1977), its possible synaptic function in invertebrates and its heterogeneous distribution in mammalian brain (Harmar & Horn, 1976), we were interested in determining whether receptors sensitive to this amine exist which differ from noradrenaline receptors on central neurones of the rat. This paper provides experimental evidence that such receptors exist and that a role of octopamine in synaptic transmission independent of catecholaminergic

mediation remains a tenable hypothesis for the mammalian CNS. Some of these results have been communicated to the Canadian Physiological Society (Hicks & McLennan, 1977).

Methods

Experiments were performed on 29 rats anaesthetized with urethane (1.5 g/kg, i.p.). Body temperature was maintained at 37.5°C by means of an electrical heating pad. Following removal of a cranial bone flap, the dura was reflected and the exposed cortical surface was covered with a pool of liquid paraffin. Amines were applied with anodic currents into the immediate extracellular environment of single neurones by electrophoretic ejection from micropipettes.

Micropipettes

On the day before each experiment, seven-barrel micropipette assemblies were filled with distilled H_2O by boiling. The pipettes were then cooled and the H_2O replaced by the solutions of drugs. The central and one outer barrel of each pipette assembly were filled with 4.0 M solutions of NaCl for extracellular recording and current neutralization respectively. The remaining barrels were filled with solutions of dopamine hydrochloride (0.5 M, pH 3.5 to 5.0), α-flupenthixol (0.2 M, pH 3.4), sodium glutamate (0.5 M, pH 8.0), metoclopramide monochloride (0.2 M, 40 mM and 20 mM in 0.15 M NaCl, pH 5.8), noradrenaline bitartrate (0.5 M, pH 2.7 to 5.2), octopamine hydrochloride (0.5 M, pH 3.0 to 5.2) and propranolol (0.2 M, pH 3.4). In all cases at least 18 h were allowed for drug diffusion before the pipettes were used. Electrode assemblies were broken to give tip diameters of 5 to 10 μm and resistances of the recording barrel of 1.5 to 5.0 MΩ. The drug-containing barrels were checked for adequate current flow before each experiment.

Recording and drug administration

Neuronal responses were monitored on an oscilloscope equipped with a variable pulse discriminator and were recorded by means of a ratemeter and moving chart paper. When cells were encountered which responded to octopamine and/or catecholamines, the drugs were sequentially ejected for fixed durations and at fixed intervals with currents that elicited submaximal effects. Between applications of drugs retaining currents of −10 nA were passed. In some cases, drugs could be administered systemically through a cannula placed in the femoral vein. In any experiments where the spike amplitude changed during the course of the application of drugs, the results were discarded.

Results

Effects of octopamine and noradrenaline

Both increases and decreases in the rates of firing of cerebral cortical neurones were observed in response to the amines. From a sample population of 95 unidentified cortical neurones, noradrenaline decreased the rate of firing of 78% (74 cells) and had an excitatory effect on 14% (13 cells) (Table 1). The action of octopamine upon the same neurones was different, as it depressed 48% (46 cells) and excited 42% (40 cells) and had no effect on the remaining 9. An analysis of contingency (Table 1) by the χ^2 test indicates an insignificant association between the effects of octopamine and noradrenaline ($P > 0.36$). Of particular note is the observation that on 34 neurones the two amines had opposite effects. In Figure 1a the responses of a cell which was depressed by noradrenaline and excited by octopamine are shown. It was also observed that depressions elicited by noradrenaline often considerably outlasted the appli-

Table 1 Numbers of unidentified cortical neurones tested with both octopamine and noradrenaline ($n = 95$)

		Octopamine		
		Excitation	*Depression*	*No effect*
Noradrenaline	*Excitation*	7	6	0
	Depression	28	37	9
	No effect	5	3	0

χ^2 analysis of these results showed no statistically significant association between the effects of these two amines ($P > 0.36$).

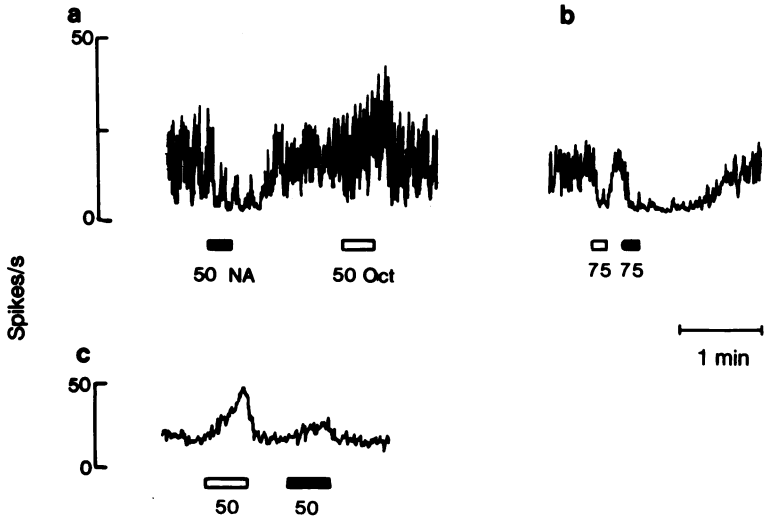

Figure 1 Examples of the effects of octopamine and noradrenaline on the firing rates of neurones of the cerebral cortex. Ordinate scale: firing rate in spikes/s; time scale: 1 min. Horizontal bars indicate microelectrophoretic drug applications, numbers correspond to intensities of ejecting currents ($\times 10^{-9}$A). (a) A neurone responding with inhibition and excitation to application of noradrenaline (closed bars, NA) and octopamine (open bars, Oct) respectively. This cell was located 1.15 mm below the surface of the cortex. (b) Another neurone (depth 1.34 mm) responding with depression to both octopamine and noradrenaline. Note the difference in duration of effect between the two amines; ordinate scale as in (a). (c) Neurone responding to octopamine with a stronger excitation than to noradrenaline (depth 1.5 mm).

cation of the substance, and the duration of the after-effect depended on the ejecting current. By contrast the depressions caused by octopamine typically lasted only as long as the expelling current continued (Figure 1b). The responses were unaffected by the pH of the solutions of amines over the range used (cf. Frederickson, Jordan & Phillis, 1971).

On 26 of the 37 cells which had their firing rates decreased by both amines, it was observed that octopamine was of less apparent potency as a depressant than was noradrenaline, judged by the intensities of the ejecting currents required to elicit effects of equivalent magnitude. The two amines appeared to be of similar potency in lowering the rates of firing of the other 11 neurones. By the same criterion octopamine was apparently more potent than noradrenaline in increasing the neuronal firing rate of 6 cells: an example is shown in Figure 1c. When this type of action occurred, it was also observed that other neurones tested with the same electrode could yield more powerful noradrenaline-evoked depressant responses. Electrode artifact is therefore unlikely to explain the illustrated result. Accurate quantitative measures of potency cannot of course be obtained without a knowledge of transport numbers for the ejected compounds.

Effects of dopamine

On most cells which were responsive to octopamine, dopamine was tested to examine the possibility that the effects of the phenolic amine might be due to interaction with a dopamine receptor. The effects of octopamine also showed no correlation with those of dopamine (Table 2; χ^2 test, $P > 0.20$), as was the case with those of noradrenaline.

Dopamine-elicited changes in firing rate were of short duration, usually lasting only as long as the application of the amine. Of 74 cells depressed by noradrenaline, 48 were depressed by dopamine and 19 were excited (Figure 2). Of these 19 cells, 10 were excited and 6 depressed by octopamine, with the remaining 3 unaffected. Of the 13 cells excited by noradrenaline 9 were also excited by dopamine and the other 4 cells did not respond.

Identified cortical neurones

In an attempt to identify those cortical neurones which were affected by low doses of the amines, stimulating electrodes were stereotaxically positioned in the crus cerebri (A 3.2, L 2.6, V 2.6 from stereotaxic zero according to König & Klippel, 1974) and the

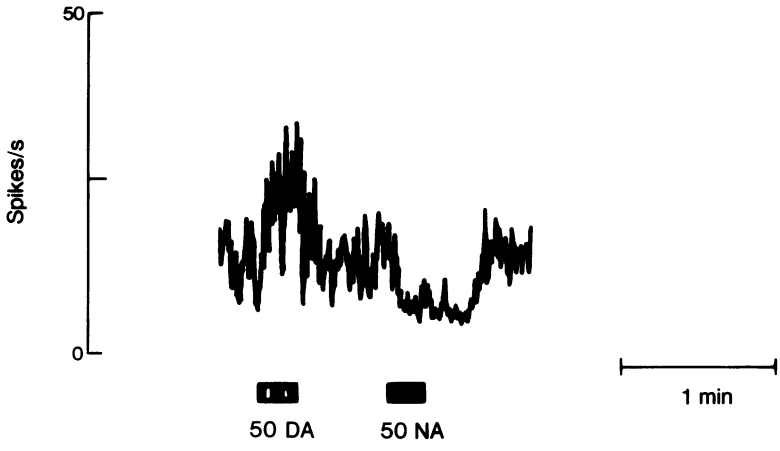

Figure 2 Responses of a cortical neurone to dopamine (divided bar, DA) and noradrenaline (solid bar, NA) (depth 0.24 mm).

ventrobasal complex of the thalamus (A 4.2, L 2.8, V 3.9). Thirty one cells which were activated by antidromic stimulation from the crus cerebri or which fired at latencies of 5 to 12 ms following thalamic stimulation were tested for their responses to electrophoretically applied amines. The responses of these two groups of identified neurones to the agonists were indistinguishable, and are presented in Table 3. The pattern of response also appears not to be markedly different from that of the unidentified cells of Table 1. However, on all of the identified cells high ejection currents (up to 100 nA) were required to obtain even minimal effects with octopamine.

Effects of propranolol and α-flupenthixol

In order to determine more specifically the independence of octopamine-induced changes in firing rate from those of the catecholamines, an investigation of the effects of adrenoceptor and dopamine receptor antagonists was conducted. Propranolol was effective as a blocker of the depressant responses of noradrenaline at doses which left octopamine-elicited increases in firing rate unaltered. Since this compound has local anaesthetic properties (Engberg & Ryall, 1966; Johnson, Roberts, Sobieszek & Straughan, 1969; Stone, 1973) complete tests were achieved on only 4 neur-

Table 2 Numbers of unidentified cortical neurones tested with both octopamine and dopamine (n = 68)

		Octopamine		
		Excitation	*Depression*	*No effect*
Dopamine	*Excitation*	6	10	4
	Depression	19	13	8
	No effect	6	2	0

χ^2 analysis of these results show that the effects of these two amines are statistically independent ($P > 0.20$).

Table 3 Numbers of identified cortical neurones tested with both octopamine and noradrenaline (n = 31)

		Octopamine		
		Excitation	*Depression*	*No effect*
Noradrenaline	*Excitation*	4	1	3
	Depression	8	6	8
	No effect	0	1	0

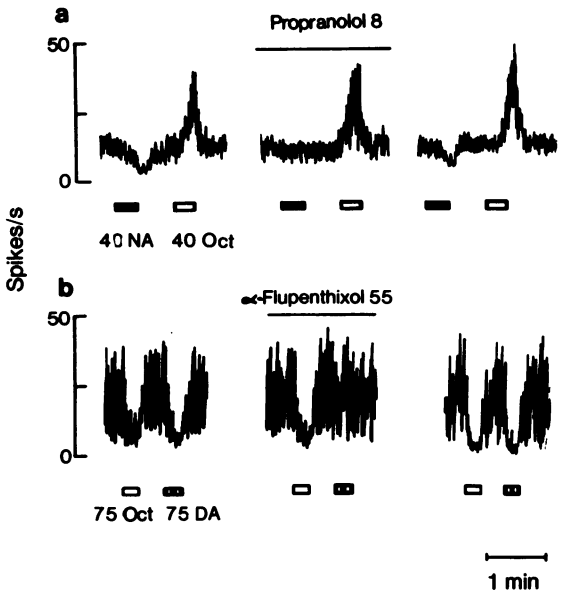

Figure 3 Responses of two neurones tested with octopamine (open bar, Oct) and noradrenaline (solid bar, NA) in (a), and octopamine and dopamine (divided bar, DA) in (b), in the presence of antagonists of the amines. In (a), antagonism by propranolol (8 nA) of the depressant effect of noradrenaline applied with 40 nA of electrophoretic current is observed while the excitatory responses caused by octopamine (40 nA) remain unaffected. Recovery was obtained 2.5 min after cessation of the propranolol administration. Neurone was located 0.96 mm below the surface of the cortex. (b) Shows the selectivity of α-flupenthixol (55 nA) in reducing the depressant effect of dopamine (75 nA) and not that of octopamine (75 nA). The responses recovered to control levels approximately 5.5 min after the α-flupenthixol (cell depth 0.45 mm).

ones (of 15 tested); however, in all attempts where recovery of the responses to control levels was achieved, selectivity of effect against noradrenaline and not of octopamine was observed (Figure 3a).

Acceptable records were obtained from 5 neurones tested with the dopamine receptor blocker, α-flupenthixol. On all 5 cells the effects of dopamine were attenuated while those of octopamine were spared. Of these 5 cells, 3 responded with depressions to both octopamine and dopamine (Figure 3b), one other was depressed by octopamine and excited by dopamine while the remaining cell was excited by octopamine and depressed by dopamine.

Effect of metoclopramide

Whether ejected electrophoretically as a cation (14 cells) or administered intravenously (500 μg/kg) (4 cells), metoclopramide showed no antagonistic action toward any of the amines on the cells examined. With both routes of administration metoclopramide caused an increase in the spontaneous rate of firing of neurones within 30 s, but the responses to administration of the amines superimposed on this enhanced background activity was unaffected. Metoclopramide caused an abrupt decrease in neuronal firing if administration was continued for 5 or 6 min (or a shorter time if higher doses were used) with recovery occurring as long as 30 min later.

Discussion

Several conclusions can be drawn from these results. Octopamine is pharmacologically active in the mammalian central nervous system, both as an excitatory and a depressant agent, and has certain actions which cannot be explained by the 'false transmitter' hypothesis (Fischer *et al.*, 1965; Kopin, 1968). A false transmitter as defined by Kopin (1968) exerts its action on the receptor which is specific for the endogenous transmitter, thereby decreasing the efficacy of synaptic transmission at the effector site. The false transmitter should therefore produce a response which is both qualitatively similar to that exerted by the normal transmitter and is blocked by antagonists of that substance. Noradrenaline and dopamine are generally considered to be active as synaptic transmitters in the mammalian CNS, and both are believed to be contained in fibres which innervate the cerebral cortex (Phillis, 1970). Since octopamine effects are not significantly correlated with those of either of the catecholamines nor are they affected by adrenoceptor or dopamine receptor blocking drugs, it seems reasonable to ascribe to octopamine a pharmacological role other than as a false transmitter (see however, Kostopoulos & Yarbrough, 1975). Indeed the fact that on many occasions actions of octopamine opposite to those of noradrenaline and dopamine were obtained is indicative of a direct effect on receptors. If the action of octopamine were indirect, i.e. to cause a presynaptic release of catecholamines, then again the effects observed with octopamine should be correlated with those of noradrenaline or dopamine.

The results obtained with the catecholamines on cortical cells antidromically activated by pyramidal tract stimulation or synaptically by stimulation of the ventrobasal thalamus are in general accord with those of Stone (1973). This author found that most of these cells were depressed by noradrenaline, that the effects were slow in onset and relatively prolonged (Stone,

1978) and that propanolol was a selective blocker of the depressions. One difference between the present results and those of Stone is the observation that pyramidal tract cells were occasionally excited when high ejecting currents for noradrenaline were used, while Stone did not describe such noradrenaline-induced excitations.

The results with two antagonists offer compelling evidence that octopamine receptors exist which are distinct from those used by the catecholamines. Propanolol and α-flupenthixol respectively prevented noradrenaline- and dopamine-induced changes in the firing rate confirming, in part, similar results by Bradshaw, Bevan & Szabadi (1978). Neither of these compounds reduced the effects elicited by octopamine, suggesting that receptors mediating the actions of octopamine are pharmacologically distinct from those of the catecholamines. The results confirm and extend the observations of Giardina, Pedemonte & Sabelli (1973), who reported opposite effects of phenylethylamine and phenylethanolamine when compared with noradrenaline on cortical neurones.

Metoclopramide, a chlorbenzamide derivative with anti-emetic and cataleptic properties (Ahtee, 1975; Jenner, Marsden & Peringer, 1975) and which has been described as an effective antagonist on the stereospecific receptors for octopamine in *Tapes watlingi* (Dougan, Wade & Mearrick, 1975; Dougan & Wade, 1978), has no effect as an antagonist for any of the amines tested in the cortex of the rat: indeed it has powerful actions of its own on those neurones examined.

It is difficult to evaluate in terms of the present results, the significance of a function for octopamine as a synaptic activator which has been proposed by Boulton (1976). If octopamine does 'set the tone', or 'prime' the postsynaptic membrane of noradrenergic synapses, then unless octopamine can both increase and decrease the probability of transmission at these junctions between different cells, a role solely as a modulator of synaptic actions seems unlikely. Caution is further warranted before accepting the view that octopamine is a synaptic activator, since the effect of octopamine appears occasionally stronger than that of noradrenaline, at least so far as cortical excitations are involved.

Further experiments will be required before it can be concluded that octopaminergic transmission occurs in mammalian nervous tissue. However, the results described here do offer experimental support for the existence of receptors sensitive to octopamine which are independent of those receptive to the catecholamines, and it is conceivable that they may be involved in separate synaptic processes.

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