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Effect of cyproheptadine on the octopamine-induced responses in the mammalian central nervous system[1]

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Summary. Extracellular recordings have been made from rat thalamic neurones anaesthetized with urethane, 1.5–2 g/kg i.p. Iontophoretically applied octopamine excited certain thalamic neurones in the ventral basal complex while inhibiting others. Both effects were reversibly antagonized by iontophoretically applied cyproheptadine without affecting responses to noradrenaline and dopamine.

There is now considerable evidence for a role for octopamine as a transmitter or modulator in the invertebrates[2–4]. Octopamine has been found to have an uneven distribution in the mammalian brain[5] and its levels can be manipulated following drug pretreatment[6]. Octopamine has also been shown to have actions on rat cortical neurones which would appear to be distinct from a possible effect on noradrenaline or dopamine receptors[7]. These authors reported that propranolol and α-flupenthixol specifically blocked noradrenaline and dopamine induced responses respectively on rat cortical neurones, without having an effect on octopamine responses. Metoclopramide had no effect on any of the responses to these amines. However we have found that none of these antagonists were of use in differentiating between octopamine and dopamine or noradrenaline-induced responses to rat thalamic neurones in the ventral basal complex. During an investigation to try and find a specific octopamine antagonist we have found that cyproheptadine can selectively and reversibly block the inhibitory or excitatory actions of octopamine on thalamic neurones within the ventral basal complex without affecting either noradrenaline or dopamine responses.

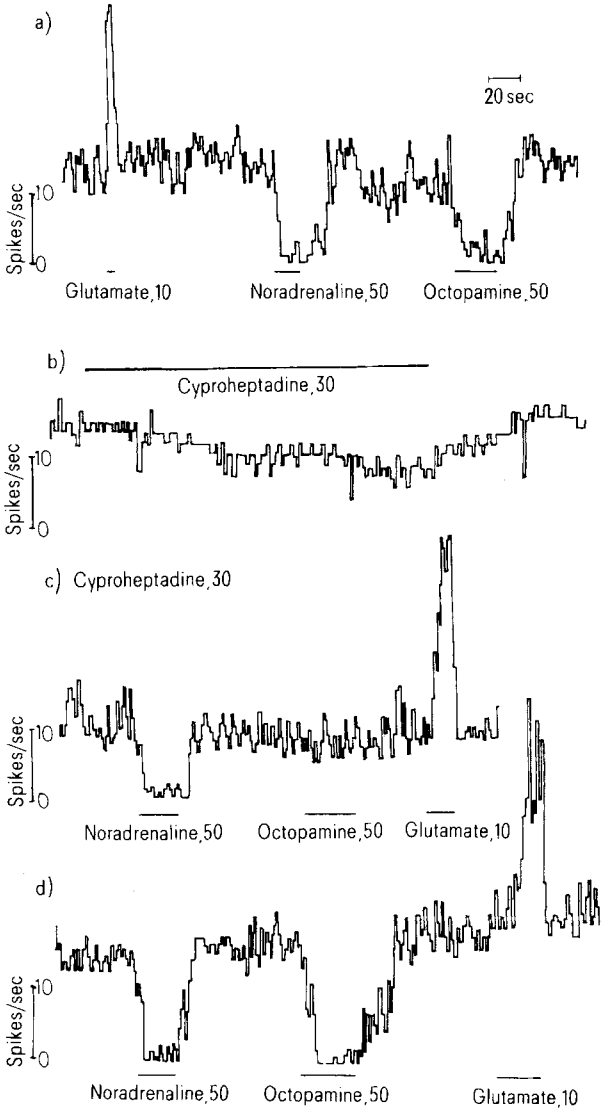
Materials and methods. Experiments were performed on 15 male Wistar Albino rats weighing 200 g and anaesthetized with urethane 1.5–2 g/kg i.p. Extracellular recordings were made from single neurones in the ventral basal complex of the thalamus using parallel multibarrel glass microelectrodes[8]. Cells were identified by stimulating the locus coeruleus using a concentric bipolar stimulating electrode. The position of the neurones was located by ejecting Pontamine Sky Blue from the recording barrel[9] and then preparing frozen sections. The drugs in this study were iontophoretically ejected from the multibarrel microelectrode. DL-Octopamine, dopamine, (−)-noradrenaline and 5-hydroxytryptamine, all at 0.5 M, were ejected as cations, as was cyproheptadine hydrochloride, 3 mM. L-Glutamate, 0.5 M was ejected as an anion. Current balancing was used during the ejection of all drugs[10].

Results and discussion. Cyproheptadine was tested on the responses to noradrenaline and octopamine on 18 neurones in the ventral basal complex of the thalamus. Octopamine, 30–70 nA, was reversibly blocked by cyproheptadine, 5–40 nA, on all 18 different thalamic neurones where cyproheptadine had no effect on the noradrenaline response. The table shows in 10 cases both amines inhibited the activity of the neurone under test, in 1 case octopamine inhibited the cell while noradrenaline excited, in 4 cases octopamine excited the cell while noradrenaline inhibited and in 3 cases both amines excited the neurones. The figure shows the result of an experiment where both amines inhibited cell activity. Glutamate, 10 nA, excited the cell while noradrenaline and octopamine, both at 50 nA, inhibited cell activity (figure, a). In the presence of cyproheptadine, 30 nA for a total of 5 min, there was no effect on the noradrenaline and glutamate responses while the octopamine response was completely blocked (figure, c). In the figure, d, can be seen the recovery of the octopamine response. It took 6–7 min for the octopamine response to completely recover. Note

The table shows the effects of 18 thalamic neurones in the ventral basal complex tested with both octopamine and noradrenaline. All the octopamine responses were reversibly blocked by cyproheptadine (30 nA) where cyproheptadine had no effect on the noradrenaline responses

	Noradrenaline Excitation	Inhibition
Octopamine Excitation	3	4
Inhibition	1	10

that cyproheptadine had a depressant effect on the firing rate of the cell (figure, b). In a separate series of experiments in the ventral basal complex where 3 cells were excited by dopamine and octopamine, cyproheptadine blocked octopamine without any effect on dopamine. In 3 other neurones in the same regions where dopamine excited cell activity and octopamien inhibited the activity, again cyproheptadine reversibly blocked the octopamine response leaving the dopamine response unaltered. At currents which blocked octopamine, cyproheptadine had no effect on 5-hydroxytryptamine responses on 9 of the neurones tested in the ventral basal complex.



Rate meter recording showing the effect of cyproheptadine on the noradrenaline, octopamine and glutamate-induced responses on the firing rate of a thalamic neurone in the ventral basal complex. Ordinate scale: firing rate in spikes per sec; time scale, 20 sec. *a* Firing rate was depressed by octopamine (50 nA) and noradrenaline (50 nA) but excited by glutamate (10 nA) applied iontophoretically as indicated by horizontal bars. *b* The depressant effect of cyproheptadine (30 nA) on the firing rats of the cell. *c* In the presence of cyproheptadine (30 nA) the depressant effect of octopamine was blocked, but the noradrenaline-induced inhibition and the glutamate-induced excitation were not affected. *d* Octopamine response recovered to control levels approximately 6 min after cyproheptadine. (Cell depth was approximately 5.5 mm below the surface of the cortex.)

These results clearly demonstrate cyproheptadine, an antagonist of 5-hydroxytryptamine[11], can differentiate between octopamine and either noradrenaline, dopamine or 5-hydroxytryptamine. One problem in trying to find a specific antagonist for octopamine is the similarity between the structures of noradrenaline and octopamine and hence the similarity in the structural requirements for activation and blocking of the receptors. In invertebrate tissues the situation is easier as one is normally trying to distinguish between octopamine and dopamine responses where there is a greater difference between the two structures and presumably between the receptor requirements for activation and antagonism.

Thus, on *Helix* neurones phentolamine has been used to distinguish between octopamine and dopamine receptors[12]. While Dougan and Wade[13–15] have found that sulpiride and clozapine will reduce octopamine excitation on lamellibranch heart without influencing the dopamine excitation. Metoclopramide also blocked octopamine but not dopamine on this preparation. Harmer and Horn[16] found that phentolamine and cyproheptadine were the most potent antagonists of the insect octopamine activated adenylate cyclase system and cyproheptadine competes with octopamine for the octopamine sodium dependent high affinity uptake system in the cockroach nerve cord[17]. Phentolamine also blocks octopamine activated adenylate cyclase in the nervous system of *Limulus polyphemus*[18]. In the present experiments on rat thalamic neurones, phentolamine failed to distinguish between dopamine, noradrenaline and octopamine. From the invertebrate studies it is of interest that insect octopamine activated adenylate cyclase and high affinity uptake system can be blocked by cyproheptadine and suggests a possible similarity between the octopamine receptor at these sites and that on the rat thalamic neurones in the ventral basal complex. From this present study it is hoped that cyproheptadine may be of value in the elucidation of possible octopamine mediated pathways in the mammalian central nervous system and further studies are in progress on this.

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