HISTAMINE H_2-RECEPTORS IN THE BRAIN AND SLEEP PRODUCED BY CLONIDINE

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Sleep was induced in chicks aged 4–7 days by intravenous injection of clonidine hydrochloride 0.04 μmol/kg. Sleep was not prevented or altered by a preceding intramuscular injection of blockers of histamine H_2-receptors which were used in doses (as μmol/kg) of up to 800 (metiamide) and 2400 (cimetidine). Clonidine, therefore, does not cause sleep by stimulating H_2-receptors in the brain. The highest dose of cimetidine used had a hypnotic action of its own.

Introduction

In an earlier paper (Holman, Shillito & Vogt, 1971) an attempt was made to determine whether the sleep induced in chicks by intravenous injection of clonidine (Zaimis, 1970) can be considered to be an effect on central noradrenaline receptors. Although the results were compatible with that view, the antagonism between clonidine and blockers of α-adrenoceptors was only partial, a dose of phentolamine 40 μmol/kg reducing, but not abolishing, the effect of clonidine 0.04 μmol. This may well be due to the fact that central noradrenaline receptors differ in their properties from peripheral ones, but it left the possibility open that other receptors were involved. The work had further suggested that tryptaminergic receptors were not involved.

A recent paper (Karppanen, Paakkari, Paakkari, Huotari & Orma, 1976) suggested that some central effects of clonidine may be on histamine receptors of the H_2-type. The authors used rats anaesthetized with urethane, and by infusing the H_2-antagonist metiamide into the cerebral ventricles, reversed the hypotension produced by clonidine. To test whether sleep elicited by clonidine was similarly affected, two blockers of H_2-receptors, metiamide and cimetidine, were given to newly-hatched chicks before intravenous administration of clonidine.

Methods

Male chicks were used during the first week after hatching, when the blood-brain barrier is not yet functional. This was essential since both metiamide and cimetidine are strongly ionized and do not penetrate the barrier. One mmol of either drug was dissolved in 1 ml N HCl, 0.2 ml N NaOH added, and the mixture made up with water to 5 or 10 ml. This yielded solutions of 0.1 or 0.2M at a pH of approximately 6. They were injected into the breast muscles of both sides in volumes ranging from 0.05 to 0.2 ml; this volume was increased to 0.3 ml on 2 occasions only. The dose of clonidine, injected intravenously at varying intervals after the histamine antagonists, was kept constant at 0.04 μmol/kg. This dose (about 0.5 μg to a 50 g chick) is about twice the ED_{50} and causes sleep in practically all chicks. Sleep was defined as eye closure and complete muscular relaxation, causing the beak to hit the bench on which the animal was standing. Slight tactile stimuli caused immediate brief awakening.

Results

Both of the antagonists of H_2-receptors, when given in sufficiently high doses, were apt to cause eye closure in the chicks; this was seen with metiamide 800 μmol/kg and cimetidine 1600 μmol/kg. The effect lasted no more than a minute or two. When, however, cimetidine 2400 μmol/kg was injected, sleep leading to complete relaxation was produced, and lasted about 25 minutes. This dose (corresponding to 30 mg for a 50 g chick) was therefore the limit of the amount which could be injected; even these high doses did not cause any visible after-effects, indicating the very low toxicity of the compound.

Table 1 summarizes the results. In spite of the pretreatment with histamine H_2-antagonists, not a single chick failed to fall asleep, usually within the first minute, after the intravenous injection of clonidine; nor was the duration of sleep outside the normal limits. After three experiments with metiamide, known to be the less potent of the two H_2-antagonists, cimetidine was used for the remaining trials; 800 and 1600 μmol were injected intramuscularly followed by clonidine after intervals ranging between 2 and 64 minutes. In two experiments with 2400 μmol/kg, a hypnotic dose of cimetidine, one hour was allowed between the two injections to ensure that the chick was fully awake

when receiving the clonidine. A few experiments were carried out with low doses (10–20 μmol/kg) to exclude the possibility that cimetidine might produce a biphasic effect and have an antagonistic action at low doses only.

Discussion

The preceding results rule out the possibility that the sleep produced in chicks by clonidine is mediated through histamine H_2-receptors. Whereas phentolamine 40 μmol/kg antagonizes the hypnotic effect of clonidine (Holman *et al.*, 1971), cimetidine, even in a dose of 2400 μmol/kg, does not.

Table 1 Sleep* induced by clonidine hydrochloride in chicks (aged 4–7 days) pretreated with metiamide or cimetidine

Drug	*Dose (μmol/kg)*	*Interval (min) between H_2-antagonist and clonidine*
Metiamide	400	10
	800	8
	800	18
Cimetidine	10	17
	10	20
	20	16
	800	3
	800	14
	1600	2
	1600	13
	1600	26
	1600	34
	1600	60
	1600	64
	2400**	58
	2400**	60

All chicks were injected intravenously with clonidine hydrochloride 0.04 μmol/kg at various intervals (see column 3) after intramuscular injections of either metiamide or cimetidine. The treatment did not modify either onset or character of sleep.
The duration of sleep until the *first* awakening ranged from 2 to 6 min, which was not different from that of chicks given clonidine only.
*Sleep is defined as complete relaxation with head or beak touching the ground and eyes closed.
**This dose (605 mg/kg) on its own caused sleep for approximately 25 minutes.

What is the situation concerning other central effects of clonidine? The views originally expressed by Karppanen *et al.* (1976) that H_2-receptors mediated the hypotensive effect of clonidine have not been completely supported by a later paper from the same group (Karppanen, Paakkari & Paakkari, 1977). The authors argued that, if clonidine lowered the blood pressure by stimulating H_2-receptors, the potent agonist of H_2-receptors, 4-methylhistamine, should do the same. In fact they found that its intracerebroventricular injection did not lower, but slightly raised the blood pressure. It is, of course, conceivable that the action of 4-methylhistamine is complex and that the expected hypotension is masked by other actions of this compound. However, there have recently been other findings which throw some doubt on the explanation of the depressor effect of clonidine as a stimulation of H_2-receptors. In the cat (which might, of course, respond differently from the rat), renal hypertension was reduced by intracerebroventricular clonidine, but this effect was antagonized by phentolamine and not by metiamide (Finch & Hicks, 1976). In the rat, on the other hand, inhibitory effects of iontophoretically applied clonidine on the firing rate of deep cortical cells were found to be prevented by metiamide (Sastry & Phillis, 1977).

Furthermore, clonidine has been shown to stimulate the accumulation of cyclic adenosine 3′, 5′-monophosphate (cyclic AMP) in guinea-pig brain, an action inhibited by metiamide and therefore presumably exerted on histamine H_2-receptors (Audigier, Virion & Schwartz, 1976). However, the importance of these findings for the *in vivo* actions of clonidine is problematic: as the authors point out, the cardiovascular effects of clonidine occur at a much lower dose than the EC_{50} for accumulation of cyclic AMP. Another doubt arises from the fact that histamine-sensitive adenylate cyclase is plentiful in some regions of the guinea-pig brain, but rat brain contains very little (Hegstrand, Kanof & Greengard, 1976). It is obvious that the relation between clonidine and central histamine H_2-receptors needs further clarification; any convincing evidence of interaction would help in assigning a role to receptors the function of which is still obscure.

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