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# Sleep produced by clonidine (2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride)

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## Summary

1. The dose of clonidine (given intravenously) required to elicit sleep in the young chick is 1/25th to 1/50th of an equiactive dose of noradrenaline. The approximate ED50 is 0·01 μmol/kg. Phentolamine (10–15 mg/kg, but not 5 mg/kg) antagonizes the action of both clonidine and noradrenaline.

2. Intensive treatment with *p*-chlorophenylalanine (700 mg/kg for 3–4 days) does not prevent the hypnotic effect of clonidine in the chick, although brain 5-HT is reduced to 15% of normal. Neither is natural sleep modified.

3. Sleep after clonidine is not affected by methysergide (0·1–1 μmol/kg, i.m.), but prevented by LSD (0·1–0·3 μmol/kg). The effect of LSD is interpreted as a physiological antagonism.

4. Clonidine (50 mg/kg) injected intravenously into adult rats causes sleep which is not abolished by phentolamine (5 mg/kg) or by *p*-chlorophenylalanine in doses which interfere with natural sleep.

5. When, per kg body weight, the same dose of clonidine is injected into the lateral cerebral ventricle of rats, sleep ensues in more than half the animals, and persistent eating in about a third ; only one of seventeen rats showed no change in behaviour. Eating and sleeping remained unaltered after *p*-chlorophenylalanine. The actual dose of clonidine injected into the lateral ventricle was 0·037 μmol, amounting to about 0·15 μmol/kg or 15 times the dose required intravenously in the chick. Noradrenaline 0·15 μmol per (intraventricular) injection caused eating but no sleep, whereas higher doses produced ataxia and paresis.

6. The work suggests that clonidine does not elicit sleep by an action requiring the integrity of the 5-HT-containing neurones arising in the raphé nuclei, and that its action is not on tryptamine receptors. In the chick, sleep appears to be produced by a central sympathomimetic effect ; it is possible, but not certain, that this also holds for the rat.

7. The intravenous hypnotic dose of clonidine for the cat is about the same as that for the rat, but injection is not accompanied by signs of peripheral sympathetic stimulation.

## Introduction

In therapeutic doses of 1–2 μg/kg, clonidine (2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride, Catapres, Boehringer) has a sedative effect in man large enough to interfere with the performance of a skilled task to the same extent as a

dose of ethanol inducing a blood concentration of 0·07% (Brüner & Klein, 1968). Higher doses produce sleep, irrespective of the mode of administration, since the drug passes the blood brain barrier easily. The purpose of the present paper was 2-fold: (a) To compare the hypnotic potency of clonidine with that of noradrenaline (NA), on the assumption that they both act at the same site. This is suggested by a direct sympathomimetic effect of clonidine on peripheral $\alpha$-adrenoceptors as well as on some central adrenoceptive sites (Maskrey, Vogt & Bligh, 1970). (b) To test whether the hypnotic effect of clonidine depended on the integrity of cerebral 5-hydroxytryptamine (5-HT) containing neurones. As shown by Jouvet and co-workers (Jouvet & Renault, 1966; Delorme, Froment & Jouvet, 1966; Renault, 1967; Mouret, Bobillier & Jouvet, 1968), surgical destruction of the 5-HT containing rhombencephalic nuclei of the raphé renders cats and rats sleepless; so does prevention of 5-HT synthesis by treatment with *p*-chlorophenylalanine. Interference is initially with both paradoxical and slow wave sleep. In the present work, the effect of combining *p*-chlorophenylalanine with clonidine was therefore examined.

## Methods

### *Chicks*

Newly-hatched Ross 1 chicks (Sterling Poultry Products Ltd.) were used between the ages of 1 and 10 days. Intravenous injections were made into the jugular vein of the smallest chicks, and into either jugular or wing vein of larger birds. They were watched in daytime in bright light; controls were either uninjected or injected with 0·9% NaCl solution. The injected volumes exceeded 0·1 ml/100 g only when large doses of noradrenaline had to be tested. *p*-Chlorophenylalanine (Pfizer) was suspended in 1% Tween 80 (Sigma Chemical Co., St. Louis) and injected into the region of the breast muscle; phentolamine mesylate (Rogitine, Ciba) or methysergide bimaleate (Sandoz) was dissolved in 0·9% sodium chloride solution and given into the breast muscle, followed 11–25 min later by intravenous clonidine; lysergic acid diethylamide was injected in the same way, the solution being that prepared by the manufacturers (Sandoz).

For the estimation of 5-HT, the brains were rapidly removed from the skull and the *nucleus basalis*, the region of the highest concentration of 5-HT in the bird brain, dissected out (for details see Juorio & Vogt, 1967). When 5-HT content had been lowered by *p*-chlorophenylalanine, the tissue from three birds was pooled for each estimation; the 5-HT and 5-HIAA were estimated by the combined methods of Bertler (1961) and Contractor (1966) as described earlier (Ahtee, Sharman & Vogt, 1970).

The activity of chicks and rats was recorded in activity cages (George Washington Ltd., Blue Town, Sheerness, Kent), in which the animal moved between two plates forming part of an electric circuit; changes in capacitance between the plates produced by the movement of the animal were counted, and the counts integrated by a pen recorder.

### *Rats*

A few adult male albino rats were injected with clonidine into the tail vein, but most were fitted with a cannula implanted into the right lateral cerebral ventricle. The rats were anaesthetized with sodium pentobarbitone (50–60 mg/kg) and the

cannulae implanted according to the stereotaxic coordinates of De Groot (1959): anterior 5·4, lateral 2·0 and vertical +2·5. The cannula was a simplified version of that described earlier (Myers, Casaday & Holman, 1967): a piece was cut from the nozzle of a 1·0 ml plastic syringe barrel to form a pedestal, the base of which was 7 mm wide and the overall height 5 mm. A 15 mm length of 21 gauge stainless steel tubing bevelled to a 30° angle at one end served as the outer guide. This was cemented into the pedestal with an epoxy resin (Araldite, Ciba Ltd.) in such a way that the protruding part measured exactly 6 mm to the middle of the bevel. Each guide was fitted with a 26 gauge stainless steel stylet which extended to the middle of the bevel. An injector needle was made from a 25 mm length of 26 gauge stainless steel tubing cut off square at the ends and fitted with a stop to prevent the tip from going beyond the middle of the bevel of the implanted guide. The injector was connected by polythene tubing to a 5 microlitre syringe clamped in a stand. Injections were made either by hand or with a micrometer.

The rats, which had food pellets and water *ad lib.*, were kept individually in reversed daylight (red light from 10–22 h for the 'dark' period) so as to be able to test and observe them in daytime during their period of highest activity. The E.E.G. was recorded by a Grass polygraph in a room which was kept in semi-darkness, whereas all other observations were made in red light. Injections were not started until a week or more after implantation. Before any injection the rats were observed for at least 30 min; during these control periods, they were never seen to sleep, and they very rarely ate or drank. Each rat was then taken out of its cage and held gently while the stylet was replaced by the injector. The injection took a few seconds, after which the stylet was replaced and the rat returned to its cage. Observations were continued for 1 h or more.

After a series of injections the animals were killed with an overdose of sodium pentobarbitone, and the brain was perfused through the ascending aorta with 0·9% sodium chloride solution followed by 4% formaldehyde in saline. The position of the cannula tip was ascertained in frozen sections of 40 μm stained with Luxol fast blue.

All injections into the ventricle were made in a volume of 1 μl. Sterilized 0·9% sodium chloride solution served as control fluid; clonidine was injected in concentrations of 5, 10 or 15 mg/ml in 0·9% sodium chloride; the last two solutions are slightly hypertonic, but control injections of even greater tonicity (1·8% sodium chloride) produced no visible effect on behaviour. Adrenaline and noradrenaline were dissolved in sterile water (after addition of the equivalent amount of HCl) up to concentrations of 55 mg/ml adrenaline and 51 mg/ml noradrenaline which have the same tonicity as 1·8% NaCl.

Three adult female cats were given clonidine solutions intravenously into the forepaw.

## Results

### *Chicks*

#### *Normal birds*

Zaimis (1970) has shown that intravenous injections of clonidine into young chicks causes a brief period of sleep. Because of its rapid onset it is easy to distinguish from spontaneous sleep and our experiments were therefore started on this

species. Doses of 10·6 μg/kg (0·04 μmol/kg) invariably produced sleep which started within the first minute after injection and lasted, with interruptions, for a few minutes to about 30 minutes. The chick relaxed completely, its beak or head touching the table on which it had been standing, but when lightly touched, or stirred by a sudden noise, it opened its eyes, got up, stood erect for a short time and then dropped to sleep again. The approximate ED50 was 0·01–0·02 μmol/kg (2·6–5·3 μg/kg), the larger dose being usually required for the older chicks. The effect was very similar in appearance to that seen after adrenaline or noradrenaline. However, Table 1 shows that equipotent doses of clonidine and noradrenaline differed by a factor of 25–50 in favour of clonidine; adrenaline was intermediate in its activity. The doses of catecholamines are not very different from those reported (Dewhurst & Marley, 1965) as 'optimal doses', particularly if one considers that we used a rather insensitive method of observation, whereas Dewhurst & Marley recorded the E.E.G. Table 1 also indicates that, as the chicks grow older, relatively more NA is required per kg body weight, probably because the blood brain barrier is beginning to take effect.

The suggestion that clonidine and noradenaline react with the same receptors would be supported if their effects were inhibited by the same antagonists. The sleep elicited by catecholamines given intravenously was not inhibited by phenoxybenzamine or by ergot alkaloids (Dewhurst & Marley, 1965). However, if both the NA and phenoxybenzamine were injected directly into the posterior hypothalamus, antagonism was evident (Marley & Stephenson, 1970). Delbarre & Schmitt (1969) reported antagonism between clonidine and phentolamine when they used large doses of clonidine which abolish the righting reflex. We injected phentolamine mesylate, 10–15 mg/kg, into the breast muscle of 2 day-old chicks and 15–25 min later gave either clonidine or NA intravenously. A dose of clonidine (0·04 μmol/kg) failed to produce complete relaxation when preceded by phentolamine (10 or 15 mg/kg); clonidine (0·08 μmol/kg), which is about 4 times the ED50, sometimes allowed complete relaxation to occur but only for a much shortened period of time. Noradrenaline (2 μmol/kg), which readily sends chicks to sleep, also failed to cause complete relaxation of animals pretreated with phentolamine (10 or 15 mg/kg).

For reasons to be discussed later another series of experiments was carried out on 2 day-old chicks in which clonidine was given after methysergide bimaleate or lysergic acid diethylamide (LSD), two antagonists of the peripheral actions of 5-hydroxytryptamine. Methysergide (0·1, 0·3 or 1 μmol/kg) was given intramuscularly 10–23 min before an intravenous injection of the lowest dose of clonidine which causes sleep in practically all chicks (0·04 μmol/kg). Only one out of seven chicks

TABLE 1. *Chicks, approximate* ED50 *for sleep* produced by intravenous injection of clonidine*

| | Age (days) | μmol/kg i.v. |
|---|---|---|
| Adrenaline | 3 | 0·12 |
| | 6 | 0·25 |
| Noradrenaline | 3 | 0·25 |
| | 6 | 1·00 |
| Clonidine | 3 | 0·01 |
| | 8 | 0·02 |

* Sleep is defined as complete relaxation with head or beak touching the ground.

did not sleep, and that was after the lowest dose of 'antagonist', whereas others slept after 10 times that dose.

In contrast, eight of nine chicks remained awake when LSD (0·1–0·3 μmol/kg) was injected 11–18 min before the clonidine; a single chick given double the dose of clonidine showed a long delay in the onset of sleep. Not all manifestations of 'sleep' were abolished by the antagonist: most chicks closed their eyes, but their muscles failed to relax.

*Chicks pretreated with* p-*chlorophenylalanine*

In the next group of experiments, clonidine was injected after intensive treatment of the chicks with *p*-chlorophenylalanine, starting when they were 1 day old and continued for 2–4 days. Table 2 shows the loss of brain 5-HT, estimated in the *nucleus basalis*, after increasing doses of *p*-chlorophenylalanine. Injections of 700 mg/kg for 3 or 4 days caused a fall in 5-HT to between 14 and 20% of normal, a fall which is similar to that seen in rats given a single dose of 320 mg/kg. The reduction in the concentration of 5-hydroxyindole acetic acid was greater than that of 5-HT, and the amounts were often so low that they were below the threshold of the method.

Of eighteen chicks, most of which were treated with three to four injections of *p*-chlorophenylalanine (700 mg/kg), twelve fell asleep with clonidine as rapidly and were as fully relaxed as simultaneously injected controls. In six chicks, either a somewhat longer time elapsed till they fell asleep or the posture was not completely relaxed, yet even these chicks had several minutes of what looked like deep sleep.

The natural sleep pattern of chicks treated with *p*-chlorophenylalanine was then observed when no clonidine had been injected. It became apparent that they slept spontaneously, even during daytime, and that their sleeping posture during natural sleep was indistinguishable from that of the normal controls. In order to test whether the duration of sleep was reduced, their motor activity was recorded by keeping the chicks in activity cages: records were obtained by placing single one-day old chicks into such a cage, and either injecting one of a pair while continuing the records on both chicks, or injecting both chicks and comparing the records before, during and after the *p*-chlorophenylalanine treatment which was usually continued for 3 days. Activity fell to very low levels during the hours of darkness, and no consistent differences between controls and injected animals were observed. With these, admittedly, simple methods we were thus unable to detect any alteration, by intensive treatment of chicks with *p*-chlorophenylalanine, either of natural sleep or of the sleep-inducing effect of clonidine.

TABLE 2. *5-HT in* nucleus basalis *of chick brain 1–2 days after the last of a series of injections of* p-*chlorophenylalanine*

| Age at death (days) | Single dose (mg/kg) | No. of injections | % 5-HT remaining | No. of experiments |
|---|---|---|---|---|
| 4 | 350 | 2 | 47·4 | 2 |
| 5 | 350 | 3 | 28·1 | 4 |
| 5 | 600 | 4 | 13·7 | 2 |
| 5 | 700 | 4 | 14·8 | 2 |
| 8 | 700 | 3 | 20·0 | 2 |

Control value 0·73±0·04 μg/g. Suspensions of the drug were injected into the region of the breast muscle.

### *Rats*

The work was then continued on rats because these are made insomniac by *p*-chlorophenylalanine (Mouret *et al.*, 1968), and the effect of clonidine should disappear or be reduced if it is dependent, as is spontaneous sleep in this species, on a normal 5-HT content of neurones originating in the raphé nuclei.

#### *Intravenous clonidine*

A few preliminary experiments were made with intravenous injections of clonidine. The response was much slower in onset than in the chick and somewhat complicated by exophthalmos and other signs of peripheral sympathetic stimulation. The rats curled up into a sleeping posture about 3 min after the injection of clonidine (50 μg/kg i.v.), but their eyes remained wide open. In another group of rats, injections of clonidine were repeated as before, but preceded by phentolamine (5 mg/kg i.p.) a few minutes before the clonidine. All signs of peripheral sympathetic discharge were abolished, and the rats, as without phentolamine, spent about 30 min curled up and quiet, in 'sleeping' posture but with eyes open. The injections of phentolamine and clonidine were then repeated in rats which had been given *p*-chlorophenylalanine (315 μg/kg i.p.) 3 days previously. The sleep response to clonidine was unaltered at a time when the content of 5-HT in the tissue was at its lowest.

#### *Injections into the lateral ventricles*

The complication of rather slow onset of 'sleep' and the necessity of giving phentolamine to reduce peripheral effects suggested the replacement of intravenous by intraventricular injections. To reduce the likelihood of spontaneous sleep, observations were made in red light on rats kept in 'reversed daylight', that is in bright artificial light at night and in 'darkness' (red light) during the day. Injections could then be given in daytime during the period of highest activity of the rats. A clear response to intraventricular injections of clonidine required the same dose as was needed intravenously, and 10–30 times as much drug as in the chick (usually

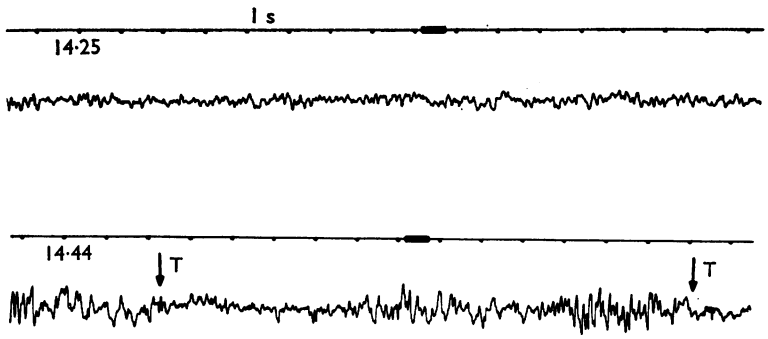


FIG. 1. Electroencephalogram showing the effect of clonidine on the rat. Upper tracing before, lower tracing 11 min after the injection into the cerebral ventricles of clonidine (10 μg). The rat's head was drooping and touched the floor of the cage 4 min after the injection and the first spindle appeared 1 min later. Light touching of the rat with a pencil is indicated by ↓T and followed by arousal.

10 $\mu$g per injection with a range of 5–15 $\mu$g, or 25–75 $\mu$g/kg). After a latency of a few minutes, most rats sought out a corner of the cage, their heads dropped until they touched the floor of the cage, and the animals remained quiet but arousable by touch or noise. Their eyes were never closed. Records of the E.E.G. were taken in order to see whether, in spite of the eyes remaining open, sleep patterns were produced. A characteristic example is shown in Fig. 1: the upper tracing was made before the injection, the lower record started 11 min after 10 $\mu$g of clonidine. Slow-wave patterns are in evidence, and were easily interrupted by an arousing stimulus, the light touch of a pencil marked by T. The first 'spindle' had appeared 5 min after the injection.

About one-third of the rats did not sleep after clonidine, but spent periods of 2–40 min eating continuously, an effect which occurs after intrahypothalamic or intraventricular injections of noradrenaline (Grossman, 1960; Myers & Yaksh, 1968). There was occasionally a mixed effect, periods of eating alternating with periods of sleep. Sleeping tended to predominate over eating with larger doses, and eating to be more pronounced with smaller ones. Only one of seventeen rats with implanted cannulae failed to show any response to 15 $\mu$g of clonidine.

The behaviour pattern which followed an injection of clonidine having been established in each individual, the rats were given *p*-chlorophenylalanine intraperitoneally (100 mg/kg on 2 consecutive days) and the experiments repeated on the third day. The response of the rats to clonidine remained unchanged: thus a rat which had slept for 7 min before *p*-chlorophenylalanine slept for 9 min one day after the second injection, while a second rat had 27 min sleep before and 35 min after treatment. Neither was clonidine-induced eating abolished. Figure 2 represents another rat treated with *p*-chlorophenylalanine before being given clonidine. An attempt was made to obtain an objective record of the effect of clonidine by placing

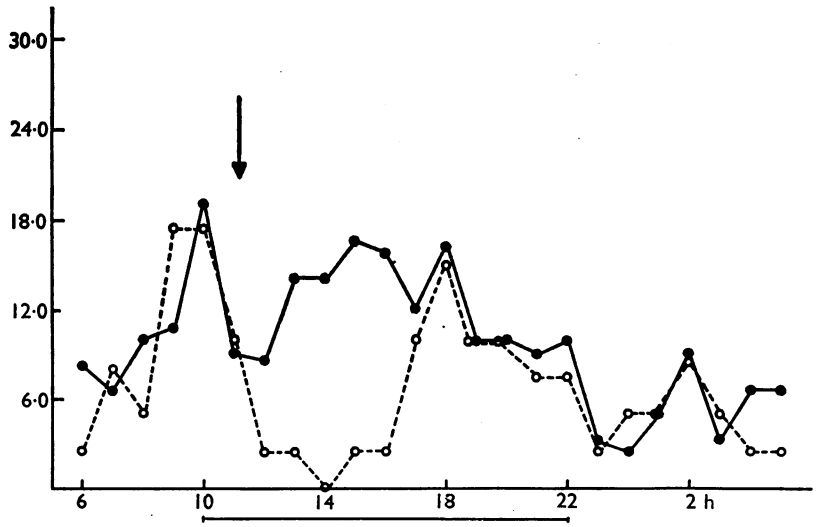


FIG. 2. Effect of clonidine on motor activity of rat. Abscissa represents a 24 h period with the hours in which the rat was kept in red light indicated by the bar. Ordinate: activity in arbitrary units (each unit representing approximately 110 movements). Continuous line: mean activity over a 3 day period; on the first of the 3 days *p*-chlorophenylalanine 320 mg/kg was given intraperitoneally to the rat. Interrupted line: day 4, with injection into the cerebral ventricles of 20 $\mu$g clonidine (in two injections given within 2 min) at the arrow.

the rat in an activity cage and recording motility. The continuous line represents the mean activity recorded on 3 consecutive days, during the first of which the rat received *p*-chlorophenylalanine (320 mg/kg). The rat was kept in darkness from 10 to 22 h and activity was high during that time. Clonidine (20 μg, double the usual dose) greatly reduced the activity for 5 h, but the tracing gives no indication of the degree of alertness or the characteristic posture of the animal: observation showed that 4 min after the injection the rat's head was resting on the floor of the cage and the animal remained so, apparently asleep, and not noticing its surroundings for about 1 hour. After that time, the rat moved little for another 4 h as shown in the tracing, but appeared awake and alert whenever inspected. The method, though demonstrating the effectiveness of clonidine after *p*-chlorophenylalanine, is unsatisfactory as a qualitative record of events.

*Comparison of clonidine and catecholamines*

The effects produced by clonidine were compared with those of injecting other substances in the same volume of 1 μl. Control solutions of sodium chloride 0·9% of 1·8% had no visible effect. The doses of catecholamines were limited to the amounts which could be given in 1 μl without exceeding twice the tonicity of blood. Adrenaline, 37·5 μg and 55 μg (the latter dose, 0·3 μmol, has the tonicity of 1·8% NaCl solution when dissolved in 1 μl), caused a loss of muscle tone and a paresis of the hind legs which lasted for 1–1·5 hours. Respiration was laboured and there was sometimes an early phase of excitement. Although the rat's behaviour did not resemble that seen after clonidine, there were periods of slow waves on the E.E.G. Noradrenaline 0·3 μmol (51 μg) also produced muscular paresis preceded by rearing, but the rat remained able to walk. Half that dose elicited eating in two rats which had slept with clonidine, and did not produce muscular disability. It was obvious that, in the rat, equipotent doses of clonidine and catecholamines for the production of sleep were unobtainable.

After the end of a series of experiments, the rats were killed and the position of the cannula was checked in frozen sections of the brain. In the experiments reported here, the cannula was always correctly placed. However, some rats which had responded consistently to injections repeated over several weeks, ceased to respond when the cannula had been in position for 6 weeks. Histological examination showed some fibrous tissue in the vicinity of the tip of the cannula which must have impeded access of fluid to the ventricular space.

*Cats*

Three cats were given clonidine intravenously to see how this species would respond. With 0·20 or 0·13 μmol/kg (53 and 35 μg/kg) the cats fell asleep within minutes of the injection, but were woken up by vomiting which took place 2–5 min after the drug. If vomiting occurred early, sleep was delayed by a few minutes. The sleeping cats were immobile, either curled up or flat on their chest, the head resting on the front paws and the eyes closed. They were more difficult to arouse during the first 15 min of sleep than later. Sleep lasted for 30 min to about 1 hour. With 0·067 μmol/kg there was vomiting followed by light sleep which was easily disturbed by noise. Uninjected companion cats kept in the same cage sometimes closed their eyes, but kept alert to their surroundings and kept their heads up. The

response to clonidine differed from that in the rat by the absence of signs of peripheral sympathetic stimulation, but the threshold dose was of the same order of magnitude.

## Discussion

The type of sleep seen after clonidine given intravenously to chicks is indistinguishable from that following the injection of catecholamines, and the ease of arousal is reminiscent of natural sleep. To obtain equal effects on blood vessels or on the nictitating membrane, the dose of clonidine has to be somewhat larger than that of NA (Kobinger & Hoefke, 1968). By contrast, the hypnotic potency of clonidine in the chick is 25–50 times that of NA: the potency ratio is thus about the same as that found for the interference with temperature regulation produced by intraventricular injection of these drugs in the ruminant (Maskrey *et al.*, 1970). The simplest interpretation would be that, in both conditions, one is dealing with central sympathomimetic effects of clonidine. The moderate antagonistic effect of phentolamine is compatible with this conclusion. So is the fact that clonidine, like NA, may elicit an eating response when injected into the lateral ventricle of the rat. However, here the analogy ends, because, whereas rats slept after clonidine, they failed to do so after the catecholamines. It may be that in the rat NA, particularly in the higher doses required for an effect, acts at more sites than clonidine, and that there is interference with sleep by its other actions, such as causing restlessness and eating.

Since it was well established (Delorme *et al.*, 1966 ; Mouret *et al.*, 1968) that *p*-chlorophenylalanine causes insomnia in the mammal by reducing the 5-HT content of the raphé nuclei, this drug was administered to chicks and to rats and the effect of clonidine examined.

The experiments on chicks were inconclusive: in spite of giving them larger doses of *p*-chlorophenylalanine than are required in mammals, neither natural sleep nor sleep after clonidine were significantly disturbed. Although brain 5-HT was low in these chicks, it may either still have been too high for failure of transmission by 5-HT containing neurones to occur, or it may be that sleep in the chick has a different neuronal basis from mammalian sleep.

Of the eighteen chicks given the combination of *p*-chlorophenylalanine and clonidine, six took somewhat longer for relaxation or did not relax their muscles as fully as controls. This may be an expression of the irritability and hypersensitivity to cutaneous stimuli which is a feature of the action of *p*-chlorophenylalanine (Tenen, 1967, 1968), and may not be related to a disturbance of normal sleep mechanisms.

None of the doubts affecting the interpretation of the experiments with *p*-chlorophenylalanine in the chick exist in the rat. The rat, too, continued to sleep after clonidine when it had been treated with adequate doses of *p*-chlorophenylalanine. Clonidine sleep, therefore, is not brought about through the intermediary of the raphé nuclei. This could either mean that sleep can be elicited by a direct action of clonidine on the receptors normally impinged upon by 5-HT containing terminals, or by an action on other, probably adrenoceptive receptors.

In the chick, it had been possible to compare the actions of clonidine and the catecholamines over a wide range of concentrations. In the rat difficulties were created by the high doses of clonidine required for sleep. The fact that, per kg

body weight, the amount of clonidine needed was the same whether it was given by vein or into a ventricle, shows the easy passage of the drug into the brain. Yet, the hypnotic dose, per kg, was 15 times as high in the rat as in the chick. When catecholamines were tried, the highest dose which could be given was 5 times the amount of clonidine used (0·3 μmol). These doses led to the development of ataxia and paresis and this fact precluded the testing of larger doses. NA (0·15 μmol) produced eating, but no sleep, and so does, according to Myers & Yaksh (1968), adrenaline in doses up to 0·22 μmol. These authors also saw the ataxia which develops on increasing the dose further. Thus it appears impossible to reproduce the hypnotic effect of clonidine by catecholamine injections into the ventricles. Whether the excitement produced by low doses, the urge to eat and, with higher doses, the respiratory embarrassment mask any tendency to sleep, cannot be ascertained. It is also possible that the site at which sleep is being elicited in the rat by clonidine is relatively inaccessible by the intraventricular route.

If clonidine sleep involves adrenoceptive receptors, stimulation in the rat must be restricted to those involved in sleep, if such receptors do indeed exist in this species, and to those mediating the eating response. On the other hand, sleep can be obtained in the rat by intraventricular injection of 5-HT (Myers & Yaksh, 1968), and it is conceivable that the receptors occupied by clonidine and responsible for eliciting sleep are tryptaminergic. Thus it seemed desirable to test the effect of substances which, at least in the periphery, are 5-HT antagonists ; their central action is, unfortunately, much more complex. The experiments were carried out on chicks and showed that sleep after clonidine was unaffected by methysergide but was prevented by LSD. Since both substances should interact with 5-HT receptors in a similar way, these results indicate that any explanation of the antagonistic action of LSD is unlikely to be one of competition for 5-HT receptors. Rather, the prevention of sleep by LSD might have been caused by its central stimulant effect, which was evident by the birds' restlessness ; this probably prevented sleep by physiological rather than pharmacological (receptor) antagonism. In contrast, the highest dose of methysergide used here, 1 μmol/kg, had, as also reported by Dewhurst & Marley (1965), no effect of its own on behaviour of the chicks.

Tryptamine receptors are thus unlikely to be involved in the sleep elicited by clonidine ; on the other hand, there is evidence for central sympathomimetic effects of clonidine in both the chick and the rat. Such effects could explain the sleep caused in the chick, but it must remain undecided whether they alone can be held responsible for the sleep produced in the rat.

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