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CENTRAL EFFECTS OF CLONIDINE 2-(2,6-DICHLOROPHENYLAMINO)-2-IMIDAZOLINE HYDROCHLORIDE IN FOWLS

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1 The effects of clonidine infused into the IIIrd cerebral ventricle, the hypothalamus or intravenously were studied on behaviour, electrocortical activity, body, comb and leg temperatures, respiration and carbon dioxide elimination in adult and young fowls (*Gallus domesticus*).

2 Behavioural and electrocortical slow wave sleep were induced by clonidine infused into IIIrd cerebral ventricle, the hypothalamus or intravenously. Surprisingly, sleep elicited by intravenous clonidine was much longer-lasting than that induced by an identical dose given intraventricularly.

3 Body temperature was lowered by clonidine given intraventricularly or infused into the hypothalamus. Depending on initial comb temperature and ambient temperature, comb temperature was elevated, unaffected or lowered as body temperature fell; temperature of the unfeathered legs also rose as body temperature declined after clonidine.

4 Following clonidine, but before any considerable decline of body temperature, tachypnoea and wing abduction developed; during recovery of body temperature, the wings were lowered and applied closely to the trunk and the feathers partly erected.

5 CO_2 elimination fell more swiftly than body temperature following intrahypothalamic clonidine in young chicks; initial recovery developed sooner than that of body temperature, but eventual recovery was delayed compared to that for body temperature. The effects of clonidine were much more marked in young chicks studied at an ambient temperature below thermoneutrality as compared to thermoneutrality.

6 The soporific effects of clonidine were attenuated by intraventricular phentolamine; its hypothermic effects were prevented by phenoxybenzamine and prevented or attenuated by phentolamine. Intraventricular atropine, haloperidol, methysergide and propranolol were ineffective.

7 Larger doses of intraventricular phentolamine elicited shivering, tachypnoea and wing abduction; body temperature was elevated, to the extent even of lethal hyperthermia. Intraventricular atropine also elevated body temperature.

8 Clonidine infused intravenously, intraventricularly or into the hypothalamus, replaced the behavioural and electrocortical arousal evoked with dexamphetamine, by sleep associated with slow wave electrocortical activity.

Introduction

Clonidine has sympathomimetic properties ascribed to an action on peripheral and central α-adrenoceptors (Nayler, Price, Swann, McInnes, Race & Lowe, 1968; Holman, Shillito & Vogt, 1971). It readily crosses the blood-brain barrier and given intravenously, induced sleep in adult rats and cats (Holman *et al.*, 1971). This was an important finding because of the controversy as to the significance of sleep produced by noradrenaline given into the brain; noradrenaline also acts on peripheral α-adrenoceptors but does not cross the blood-brain barrier, except in young chickens. Intravenous clonidine induced sleep in young chicks (Zaimis, 1970) indistinguishable from normal sleep and that elicited by intravenous noradrenaline or adrenaline (Holman *et al.*, 1971);

the hypnotic potency of clonidine was 25 or 30 times that of noradrenaline. Effects of intraventricular clonidine on body temperature of sheep and goats, exposed to different ambient temperatures, were similar to those resulting with larger intraventricular doses of noradrenaline (Maskrey, Vogt & Bligh, 1970).

In the present experiments, central effects of clonidine were studied by infusing it directly into the brain (IIIrd ventricle, hypothalamus) of young and adult fowls, and compared, where necessary, with those of noradrenaline infused into the same brain areas. Despite the similar spectrum of effects elicited by the two drugs, their profiles differed, activation of heat-loss mechanisms, for example, being much more evident with clonidine. A preliminary account of the work has been published (Marley & Nisticò, 1974).

Methods

Adult or young Rhode Island Red pullets were used. Adults (1.75-3.0 kg) were kept at room temperature (20°-25° C). Young chicks (16-18 days, approx. 85 g) were maintained at a cage temperature of 25°-31° C.

Operative and testing procedures

Recovery anaesthesia with halothane, the stereotactic implantation of infusion cannulae into the IIIrd cerebral ventricle and hypothalamus, the brain coordinates for these cannulae, implantation of a thermistor beneath the feathered skin of the interscapular region, methods for preparing and implanting cortical recording electrodes together with post-operative care, were as previously described (Marley & Nisticò, 1972). So also were methods for measuring body temperature (except in the case of leg temperature which was recorded from a thermistor taped to the unfeathered tarsometatarsal region of a hind limb), and for respiratory rate. Fowls were tested in a chamber, the ambient temperature of which was maintained at some point between 17° and 26° C (the lower and upper thermoneutral limits for adult fowls given by Barott & Pringle, 1946 and King & Farmer, 1961). For infusions via the implanted cannulae, those into the hypothalamus were in a volume of 1.0 µl or less, and those into the IIIrd cerebral ventricle of 10 µl or less. Histological preparation of the brains was also as previously described. Adult fowls were not tested until at least a week following recovery from operative procedures, and thereafter at intervals of at least a week. They were placed in the test-chamber between 9 h 00 min and 10 h 00 min and one hour's control body temperature recorded before drugs were administered.

The technique for measuring CO_2 elimination is that described by Marley & Stephenson, 1975. The chick was placed in a 1 litre chamber, maintained at 31° or 16° C, through which CO_2-free air, maintained respectively at 31° or 16° C, was passed at 1 litre/minute. Percentage CO_2 in the expired air, measured with an infra-red CO_2 analyser (Hartmann & Braun), was recorded continuously. These chickens were tested at least 24 h after operative procedures when recovery was complete; only chickens which had fed since the operation, as indicated by presence of food in the crop, were used. Ambient temperature of 31° C is within, and 16° C below the thermoneutral range for chicks of this age (Freeman, 1963; Allen & Marley, 1967); relative humidity was maintained at approximately 60%.

Drugs

The following drugs were used: the hydrochlorides of clonidine, haloperidol (Serenace, Searle), phentolamine (Regitine, Ciba) and (±)-propanolol; atropine sulphate, mebanazine oxalate and methysergide bimaleate. (−)-Noradrenaline base was dissolved immediately before use in equimolar HCl. The pH of the clonidine solutions was 6.4 to 6.5.

Results

Infusion of clonidine into the IIIrd ventricle

Behaviour and electrocortical activity Clonidine (0.02, 0.04, 0.05, 0.1, 0.2, 0.25, 0.4 µmol) induced behavioural and electrocortical sleep within 5 to 10 min of infusion and lasting approximately 20-60 min, depending on dose. This was followed by a period in which drowsy and alert states alternated (Figure 1). During sleep, fowls stood or squatted. The change from the control small amplitude, fast frequency, alert electrocorticogram (Figure 1a) to that of the larger amplitude, slow frequency sleep pattern (Figure 1b) after clonidine was associated with a two- to threefold increase of electrocortical integrals (Figure 1c). During clonidine-induced sleep, sensory stimuli induced behavioural and phasic electrocortical arousal.

Respiratory rate and wing abduction Clonidine evoked tachypnoea and abduction of the wings about 45° from the trunk, both heat-loss mechanisms. As indicated in Figure 1d, these developed within a few minutes of intraventricular

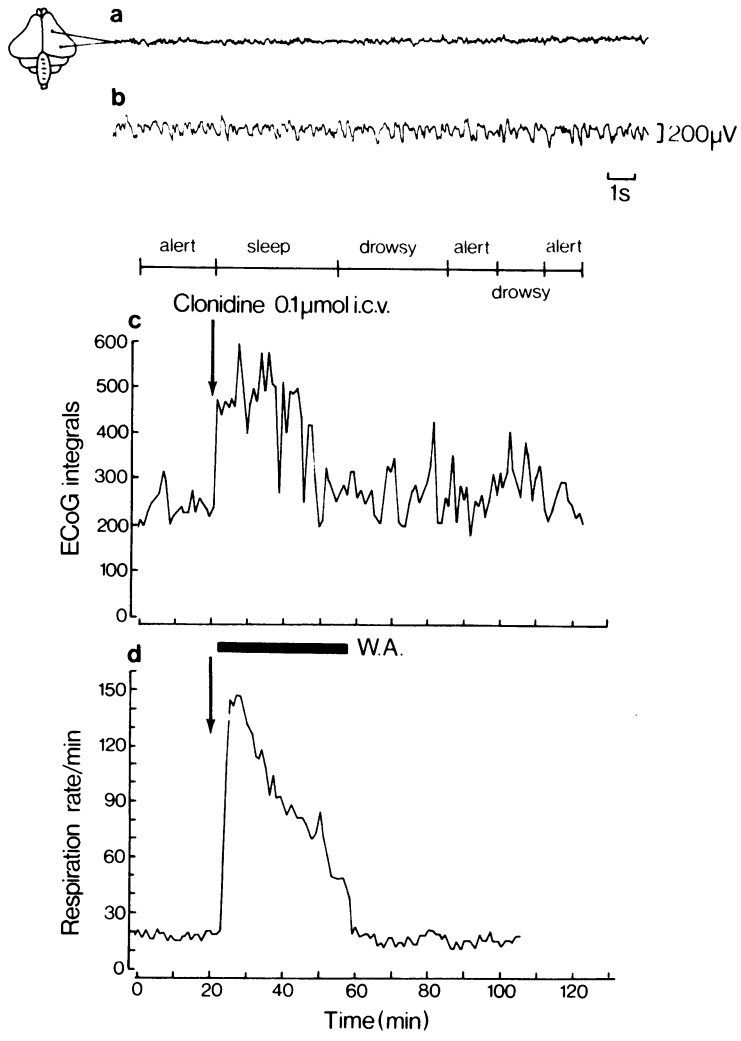

Figure 1 Records of electrocortical activity (a, b), histogram of integrated electrocortical activity (c) and graph of respiratory rate (d) in an unanaesthetized adult fowl. Solid bar indicates duration of wing abduction (W.A.). (a) Control alert electrocortical activity. (b) Slow frequency (4-7 Hz), large amplitude electrocortical potentials 15 min after clonidine, 0.1 μmol, infused into the IIIrd cerebral ventricle (i.c.v.). (c) Electrocortical integrals showing marked increase (up to a peak of 600/min) following intraventricular infusion of clonidine and associated with behavioural sleep. (d) Graph of respiratory rate illustrating increase from control values of 15-20/min up to a peak of 147/min following clonidine. Period of increased respiratory rate corresponds approximately to the duration of wing abduction.

clonidine, 0.1 μmol, a time when body temperature had fallen only 0.2-0.3°C, and persisted 40 min; tachypnoea in excess of 60-90/min was accompanied by gular flutter. These rapid respiratory rates did not disturb the induced sleep. Once wing abduction abated, the wings were lowered and applied closely to the trunk and lower limbs, the body feathers were erected and the tail flexed, procedures reducing heat loss.

Body, comb and leg temperatures Clonidine, 0.02, 0.05, 0.2 μmol, elicited dose-dependent falls in body temperature of 0.8°, 1.4°, 1.4° and 2.0°C respectively (Figure 2); duration of effect was less

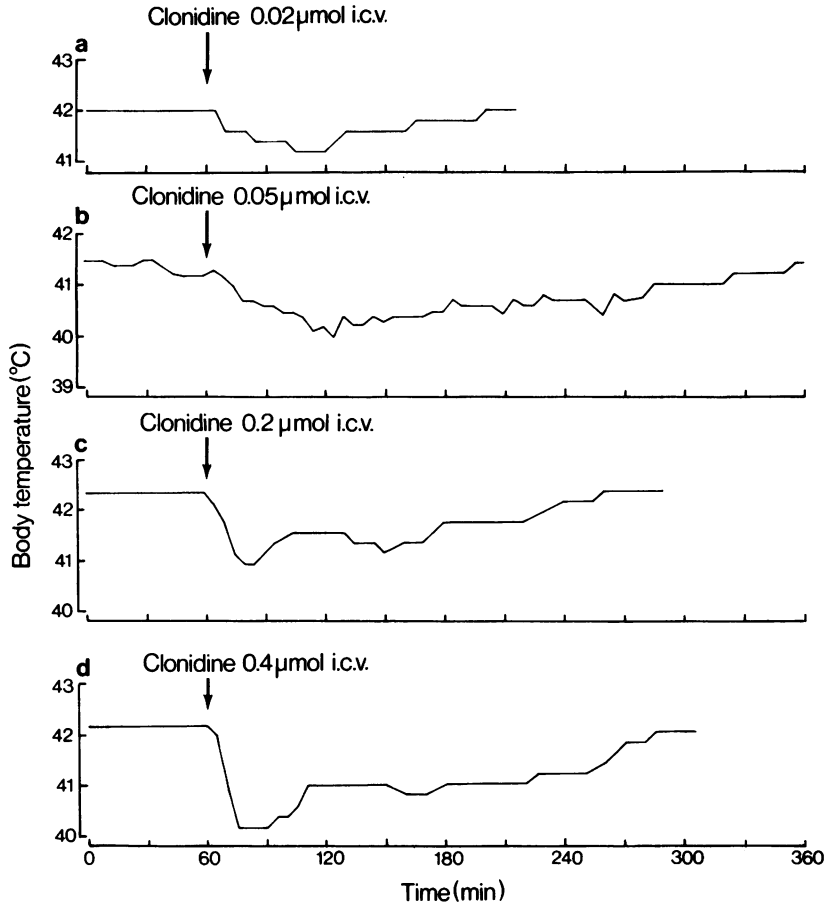

Figure 2 Records of body temperature from same unanaesthetized fowl, tested at weekly intervals, to illustrate responses to four different intraventricular (i.c.v.) doses of clonidine (0.02 μmol, 0.05 μmol, 0.2 μmol, 0.4 μmol). Ambient temperature in four experiments was 20° C.

convincingly correlated to the size of the dose. With an ambient temperature in the lower half of the thermoneutral range (17°-22° C), comb temperature was low (27°-30° C) and clonidine induced reciprocal changes in comb and body temperatures. Thus comb temperature rapidly increased by up to 10° C after clonidine 0.1 μmol, attaining its zenith before body temperature reached its nadir, the increase being maintained until body temperature recovered (Figure 3a, b). With an ambient temperature at the upper limit of thermoneutrality (25°-26° C), comb temperature was initially high (35°-37° C) and not even a large dose of clonidine (0.4 μmol), elevated comb temperature, despite lowering body temperature 1.6° C; indeed comb temperature was lowered 5°-6° C (2 of 4 fowls) or was unaltered (2 of 4 fowls).

Skin temperature of the unfeathered region of the legs Following intraventricular clonidine, 0.1 μl (2 fowls), leg temperature rose from 27°-29° C to 34°-36° C in the erect fowl, body temperature declining up to 1.4° C. However, since a similar elevation of leg temperature occurred when the fowl squatted and since sleep induced by clonidine was accompanied at some stage by squatting, this measure had limited usefulness.

Infusion of clonidine into the hypothalamus

Behaviour and electrocortical activity Eight fowls were tested and sleep consistently induced with clonidine 0.02, 0.04, 0.05 and 0.1 μmol. This developed after 5 to 10 min and lasted 30-100 min, although fowls were easily roused by sensory stimuli. Sleep was more readily induced by

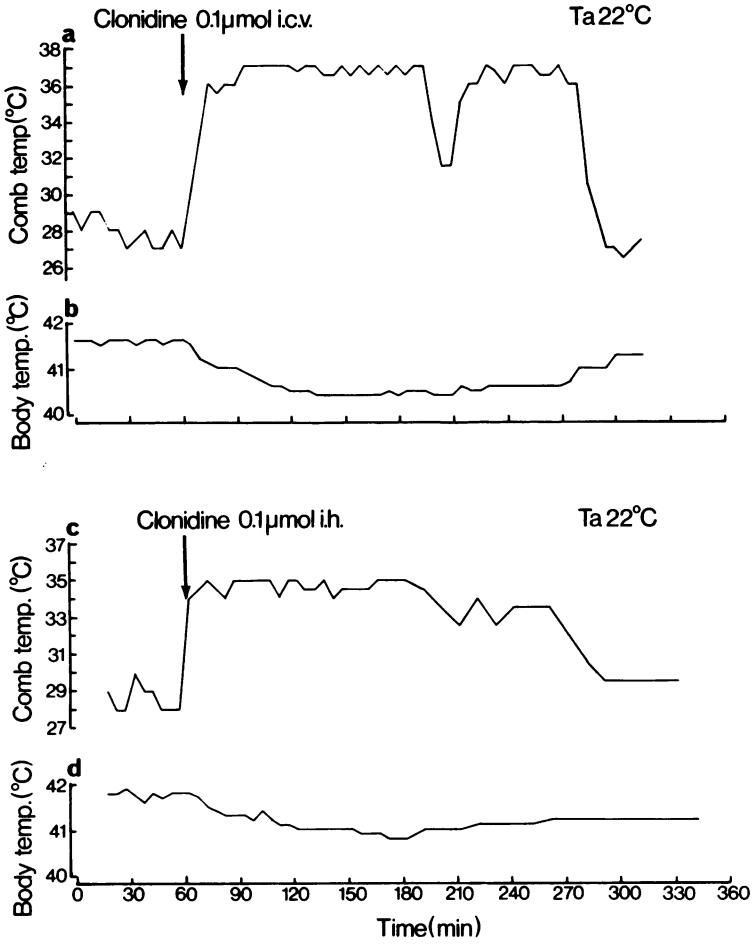

Figure 3 Records from above downwards of comb and body temperatures of two unanaesthetized fowls (a, b; and c, d respectively) to illustrate effects of clonidine infused into the IIIrd cerebral ventricle (a, b) and hypothalamus (c, d), ambient temperature (Ta) being 22° C in both experiments. (a and b) clonidine, 0.1 μmol, infused intraventricularly (i.c.v.) and in (c and d) clonidine, 0.1 μmol, infused into the hypothalamus (i.h.), promptly elevated comb temperature but lowered body temperature more slowly. Comb and body temperatures recovered *pari passu*.

infusion into the posterior rather than the anterior hypothalamus. Postural and electrocortical changes were similar to those after intraventricular clonidine.

Intrahypothalamic clonidine, unlike dopamine (Marley & Nisticò, 1972) did not elicit head movements. The soporific and hypothermic effects were not prolonged by mebanazine (100 μmol/kg i.m. 18 h, 6 h and 30 min previously).

Respiratory rate and wing abduction Within 5 min of infusion into the posterior hypothalamus of two fowls, clonidine, 0.1 μmol, increased respiratory rate, maxima of 108 breaths/min and 124 breaths/min being attained, with recovery after 50 min and 25 min respectively; gular flutter developed when respiratory rate exceeded 60/minute. In two fowls, in which the cannula-tip was more anterior, respiratory rate increased after clonidine, 0.1 μmol, but maxima of 50/min and 32/min only were achieved. Wing droop developed in the five fowls tested, preceded by wing abduction in three, the latter commencing 5-10 min after infusion and lasting 15, 45 and 70 min respectively.

Body and comb temperatures Individual maximal falls in body temperature obtained after infusing

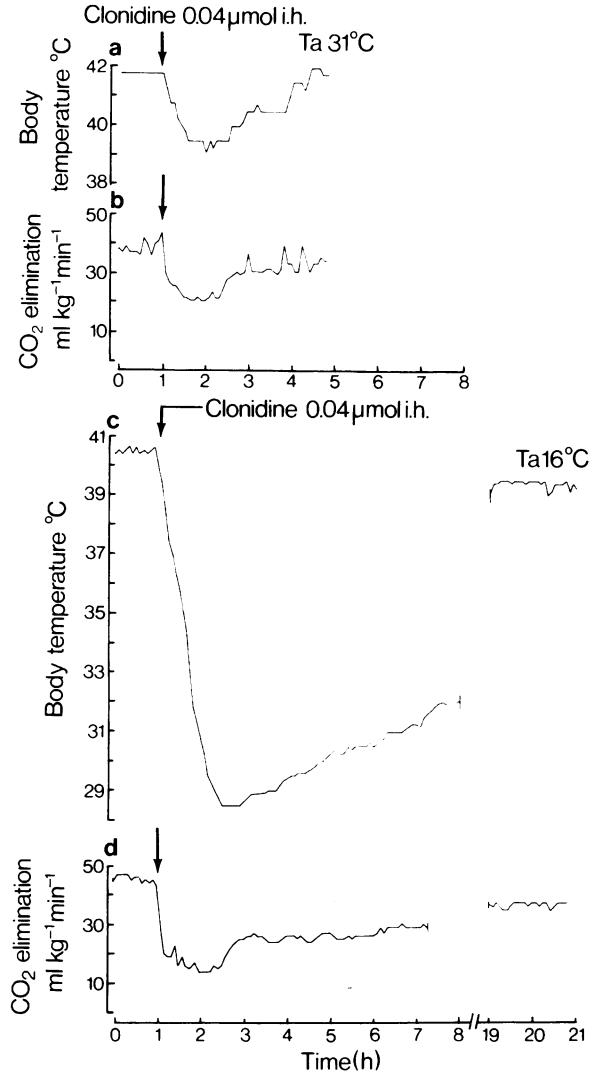

Figure 4 Effects of clonidine infused into the hypothalamus (i.h.) on body temperature and CO_2 elimination in two chicks (a, b and c, d respectively) at ambient temperatures (Ta) of thermoneutrality (31° C) and below thermoneutrality (16° C). Clonidine, 0.04 μmol, immediately lowered CO_2 elimination followed by a decline in body temperature. Onset of recovery of CO_2 elimination preceded that of body temperature although complete recovery was slower. Note effects of clonidine were much more pronounced on body temperature and CO_2 elimination at an ambient temperature of 16° C than at 31° C.

clonidine into the hypothalamus of adult fowls were as follows: clonidine 0.02 μmol, 0.6° and 1.4° C; 0.04 μmol, 1.2°, 1.5° and 1.6° C; 0.1 μmol, 0.8°, 1.0°, 1.3° and 1.7° C. Thus maximal fall of body temperature did not correlate with dose, nor did it necessarily exceed that obtained with the same dose given intraventricularly. However, whereas body temperature usually recovered within 2.5 to 4 h of intraventricular clonidine, recovery was delayed as much as 7 h with intrahypothalamic infusions, and in 3 of 4 fowls given clonidine, 0.1 μmol, body temperature was restored after 7 h only by raising ambient temperature.

With an ambient temperature in the lower half of the thermoneutral range (17°-22° C), comb

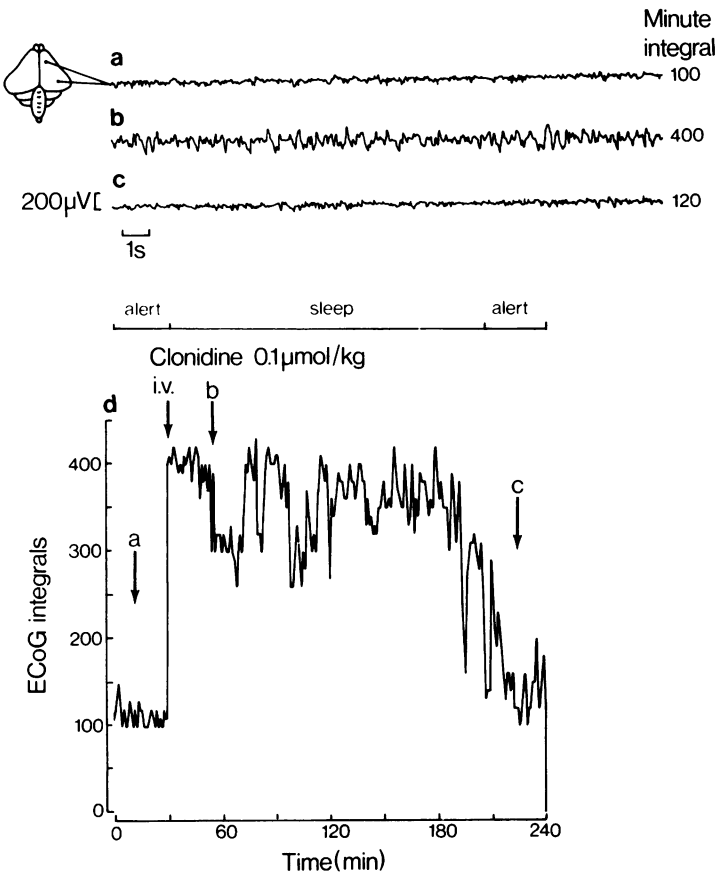

Figure 5 Records of electrocortical activity (a-c) and histogram of integrated electrocortical activity (d) in an unanaesthetized adult fowl. (a) Control alert electrocortical activity. (b) Slow frequency (4-7 Hz), large amplitude electrocortical sleep potentials 24 min after intravenous clonidine, 0.1 μmol/kg. (c) Alert electrocortical activity on recovery from sleep induced by clonidine. (d) Electrocortical integrals showing increase during sleep induced by intravenous clonidine, with subsequent reduction once sleep abated.

temperature was initially low (27°-29° C) and clonidine induced reciprocal changes in body and comb temperatures. Thus following clonidine, 0.1 μmol, comb temperature rose rapidly to 35° C (Figure 3c) as body temperature fell slowly (Figure 3d). This reciprocal relation continued during recovery. In contrast, with an ambient temperature at the upper limit of thermoneutrality (25°-26° C), comb temperature was initially high (36°-37° C) and clonidine, 0.1 μmol, despite lowering body temperature up to 1.7° C was initially without effect on comb temperature, although it subsequently fell 3°-4° C when body temperature was at its nadir.

CO_2 elimination and body temperature These experiments were performed on 16 and 18 day chicks at, and below, thermoneutral ambient temperatures. At a thermoneutral ambient temperature of 31° C (as shown in Figure 4a), clonidine, 0.04 μmol, infused into the hypothalamus lowered body temperature 2.6° C, 60 min later, with recovery at 200 min; sleep was induced for 40 minutes. CO_2 elimination declined from a mean of 39 ml $kg^{-1}min^{-1}$ in the 60 min before clonidine, to 21 ml $kg^{-1}min^{-1}$ 40 min after infusion, and recovered to a mean of 34.4 ml $kg^{-1}min^{-1}$ 2 to 3 h subsequently (Figure 4b).

At an ambient temperature below thermoneutrality (16° C), the effects of clonidine, 0.04 μmol, infused into the hypothalamus were much intensified and prolonged. Thus, maximum fall in body temperature was 11.9° C, reached

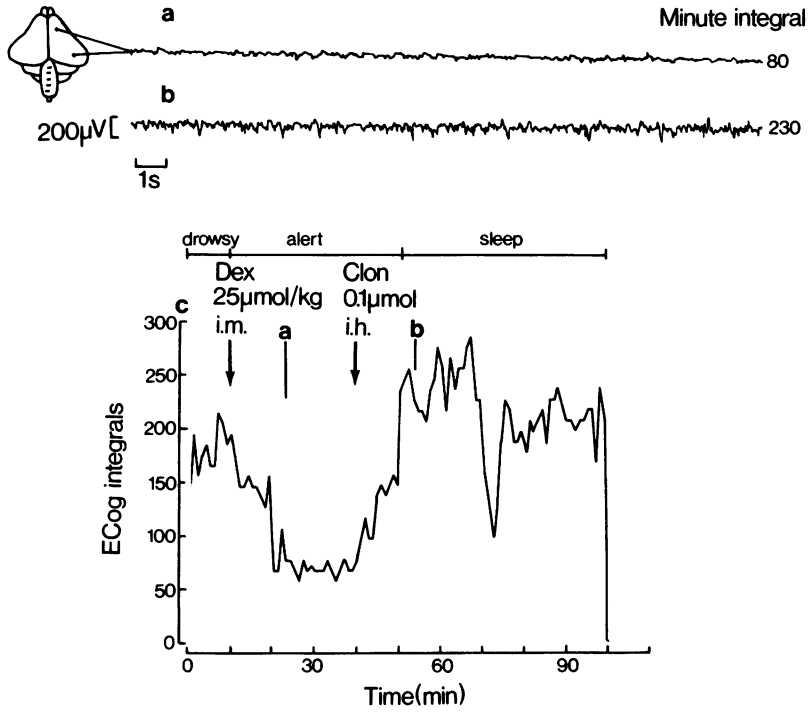

Figure 6 Records of electrocortical activity (a, b) and histogram of integrated electrocortical activity (c) in an unanaesthetized adult fowl. (a) Alert electrocortical activity induced by dexamphetamine (Dex 25 μmol/kg i.m.) injected 13 min previously. (b) Slow frequency (7-12 Hz), large amplitude electrocortical potentials, 14 min after clonidine (Clon), 0.1 μmol, infused into the hypothalamus (i.h.). (c) Electrocortical integrals showing decrease following dexamphetamine, with subsequent increase accompanying behavioural and electrocortical sleep.

90 min after clonidine and recovery was incomplete even 21 h later (Figure 4c). Mean CO_2 elimination was elevated compared to that at 31° C, averaging 45.5 ml $kg^{-1}min^{-1}$ for the control period. CO_2 elimination decreased significantly within 5 min of clonidine infusion, at a time when body temperature had fallen merely 0.3° C, and its rate of decline continued more swiftly than that for body temperature, a nadir of 14 ml $kg^{-1}min^{-1}$ being reached 60 min after infusion (Figure 4d). At 100 min after clonidine, when body temperature was at its lowest point, CO_2 elimination had begun recovery (Figure 4d), although this was still incomplete 19 h later.

Intravenous injection of clonidine

Intravenous clonidine also produced behavioural and electrocortical sleep, which were comparatively enduring. Thus intravenous clonidine, 0.05 μmol/kg (total 0.1 μmol) induced sleep lasting 90 min, whereas its duration was 40 min after clonidine, 0.1 μmol, given intraventricularly. The change from the control alert to the sleep electrocorticogram after clonidine, 0.1 μmol/kg, intravenously is shown in Figure 5a, b; electrocortical integrals increased from 100-150/min to 260-435/min (Figure 5d). Respiratory rate rose from 20/min to 122/min within 2 min of clonidine, 0.05 or 0.1 μmol/kg, intravenously and remained elevated for 6 min, subsiding to normal 16 min later.

Reversal of dexamphetamine arousal by clonidine

Clonidine given intravenously, intraventricularly, or into the hypothalamus, replaced dexamphetamine-induced behavioural and electrocortical arousal with sleep. Thus, as shown in Figure 6, once arousal had been established for 30 min by dexamphetamine 25 μmol/kg intramuscularly, and which would have customarily lasted 2 h, clonidine, 0.1 μmol, was infused into the hypothalamus; behavioural and electrocortical (Figure

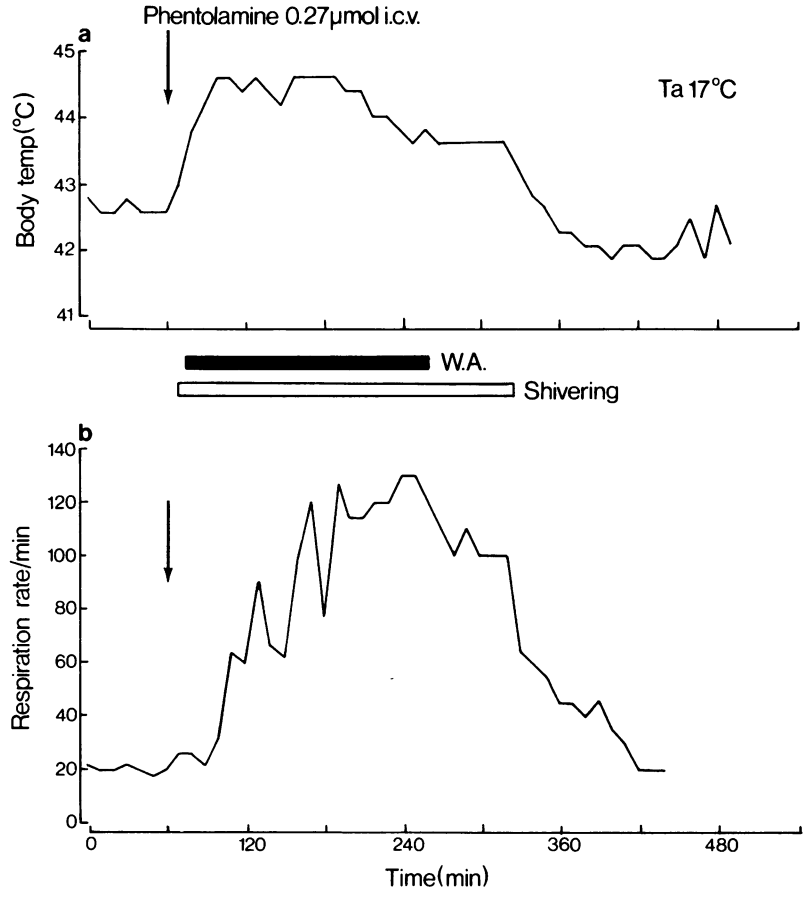

Figure 7 Records from above downwards of body temperature (a), and respiratory rate (b) in an unanaesthetized adult fowl in which phentolamine was infused intraventricularly (i.c.v.). Solid bar, and open bar, duration of wing abduction (W.A.) and shivering respectively. (a) Elevation of body temperature and (b) of respiratory rate following intraventricular infusion of phentolamine, 0.27 μmol, associated with shivering and wing abduction. Once shivering and wing abduction abated, body temperature and respiratory rate returned to pre-infusion values.

6b) sleep, accompanied by increase in electrocortical integrals (Figure 6c) developed after 11 minutes.

Antagonism of clonidine

Delbarre & Schmitt (1971) and Holman *et al.* (1971) noted that the soporific effects of clonidine were attenuated by intramuscular phentolamine. The interaction between clonidine and centrally applied phentolamine was therefore studied.

Intraventricular phentolamine was tested over a dose range 0.05 to 0.27 μmol. As indicated in Figure 7 intraventricular phentolamine, 0.27 μmol, elevated body temperature 1.9°C, palpable and visible shivering of the trunk and lower limbs commencing after about 10 min and wing abduction at 15 min; respiratory rate increased more slowly to a peak of 130/min (Figure 7b). Respiratory rate and body temperature reverted to normal, once shivering and wing abduction abated. Intraventricular phentolamine, 0.135 μmol, had similar effects, body temperature increasing 2.3°C to reach 44.5°C, but the fowl then became comatose and died. Smaller intraventricular doses of phentolamine were therefore administered before infusion of clonidine into the hypothalamus. To assess whether antagonism to clonidine was pharmacological, or merely the resultant of

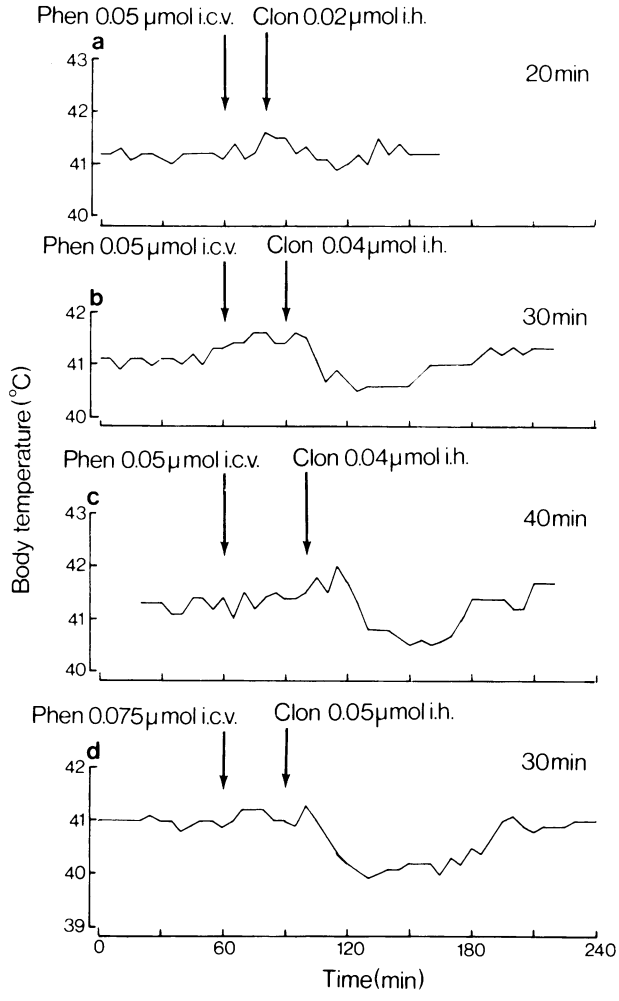

Figure 8 Records of body temperature from four adult unanaesthetized fowls in which phentolamine (Phen), 0.05 μmol or 0.75 μmol, was infused intraventricularly (i.c.v.), followed 20, 30 and 40 min subsequently by clonidine (Clon) infused into the hypothalamus (i.h.) (respectively, one dose for each fowl, 0.02 μmol, 0.04 μmol, 0.04 μmol and 0.05 μmol). There was antagonism of the hypothermic effects of clonidine 0.02 μmol. Clonidine lowered body temperature in the other three fowls, but its effects were markedly attenuated. Ambient temperature in range 21°-22° C for the four experiments.

the hyperthermic effects of phentolamine offset against the hypothermic actions of clonidine, the latter was injected in different experiments at different times after phentolamine (Figure 8). The results, except perhaps as indicated in Figure 8c in which body temperature increased 0.6°C after phentolamine, but before the hypothermic effects of clonidine developed, would argue for pharmacological antagonism. Certainly, duration of the hypothermic effects elicited with clonidine, 0.04 and 0.05 μmol (Figure 8b, d), which would otherwise have persisted for up to 7 h, were much attenuated. In confirmation, the control response to clonidine, 0.04 μmol, in the same fowl from which Figure 8c was taken, amounted to a fall in body temperature of 1.6°C with incomplete recovery 5 h after the infusion (Figure 9a), whereas following phentolamine, the same dose lowered body temperature 0.7°C with recovery 2 h after clonidine (Figures 8c, 9b). Intraventricular phenoxybenzamine, 0.25 μmol, itself without effect on body temperature, prevented

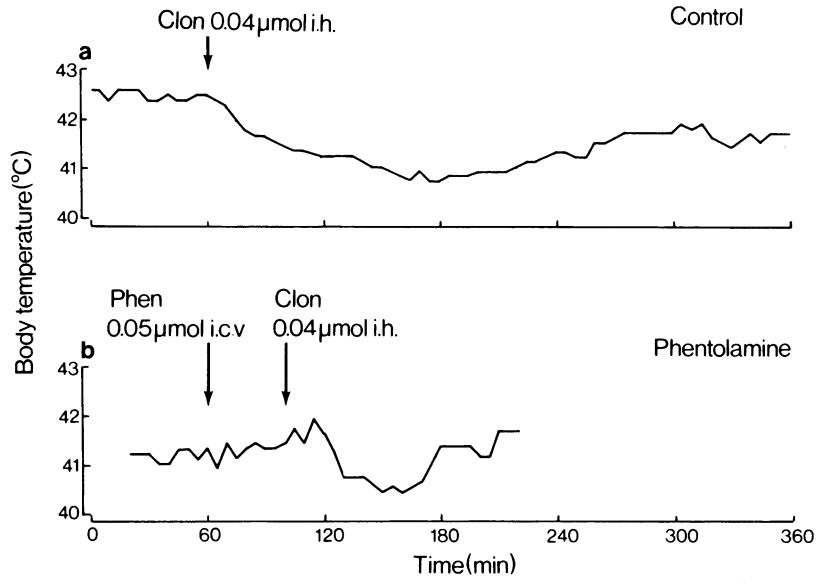

Figure 9 Records of body temperature from an adult unanaesthetized fowl. (a) Control response to clonidine (Clon), 0.04 μmol, infused into the hypothalamus (i.h.). (b) Hypothermic effect of this dose of clonidine was much reduced in duration and intensity by the intraventricular (i.c.v.) injection of phentolamine (Phen), 0.05 μmol, 40 min previously. (Experiment (b) preceded by 7 days experiment (a).)

the hypothermic effects of intrahypothalamic clonidine, 0.5 μmol. After phentolamine, sleep was not obtained with clonidine, 0.02 μmol, and was reduced in duration with the 0.04 and 0.05 μmol doses (experiments Figure 8b, d).

In other tests, antagonists were given intraventricularly 25 min before intraventricular clonidine. Atropine 0.4 μmol, haloperidol 0.4 μmol, methysergide 0.1 μmol, and propranolol 0.25 μmol, did not antagonize the soporific, hypothermic, respiratory and postural effects evoked by intraventricular clonidine 0.05, 0.08 and 0.2 μmol.

Noradrenaline

In earlier experiments (Marley & Nisticò, 1972), noradrenaline and other catecholamines, infused intraventricularly or into the hypothalamus of chickens produced sleep, diminished body temperature and evoked postural changes, amongst which lowering of the wings was conspicuous; increased respiratory rate was noted only after intraventricular isoprenaline or dopamine (see Discussion section). Noradrenaline was therefore retested, to ascertain whether tachypnoea and wing abduction were elicited and how comb and body temperatures altered.

Respiratory rate and wing abduction Following intraventricular noradrenaline, 0.5 μmol (2 fowls), respiratory rate increased from control values of 16-20/min to maxima of 56/min and 48/min respectively, the increases lasting 25 to 40 min respectively. Following intrahypothalamic noradrenaline, 0.1 μmol (1 fowl), respiratory rate increased to 124/min and was elevated for 20 minutes. Wing abduction developed in the three fowls.

Body and comb temperature With an initially low comb temperature (30° C) and an ambient temperature in the middle of the thermoneutral range (22° C), intraventricular noradrenaline, 1.0 μmol, elevated comb temperature 5.0° C (Figure 10a), with return to pre-infusion values 70 min later, body temperature falling 1.6° C (Figure 10). In contrast, when comb temperature was high (36°-37° C) and ambient temperature still 22° C, intraventricular noradrenaline, 0.5 μmol, lowered comb temperature 5° C and body temperature 1.4° C (Figure 10c, d); recovery of these temperatures occurred *pari passu*. With ambient temperature at the upper thermoneutral limit (25°-26° C) and comb temperature high (35°-36° C), noradrenaline, 0.5 μmol, infused intraventricularly or 0.1 μmol, infused into the hypo-

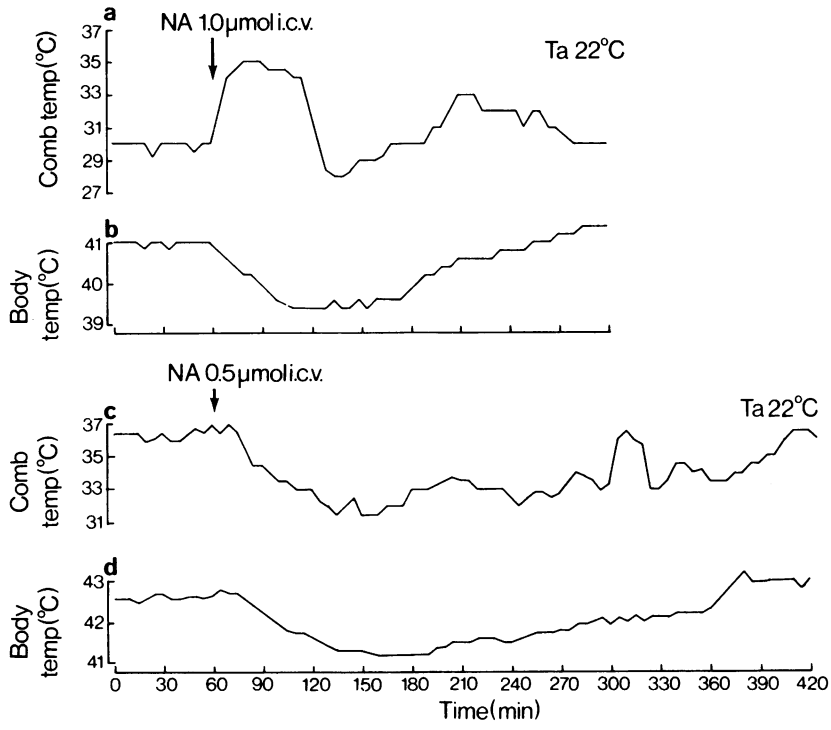

Figure 10 Records from above downwards of comb and body temperatures of two unanaesthetized fowls (a, b and c, d respectively) to illustrate effects of noradrenaline (NA) infused into the IIIrd cerebral ventricle, ambient temperature (T_a) being 22° C in both experiments. (a and b) Comb temperature initially low. Noradrenaline, 1.0 μmol, infused intraventricularly (i.c.v.) initially elevated comb temperature but lowered body temperature. (c and d) Comb temperature initially high. Noradrenaline, 0.5 μmol, infused intraventricularly (i.c.v.) lowered both comb and body temperatures.

thalamus, although depressing body temperature up to 1.6° C, scarcely affected comb temperature, lowering it 2° C at most.

Discussion

Clonidine infused into the IIIrd cerebral ventricle or hypothalamus of fowls induced behavioural and slow wave electrocortical sleep and a fall in body temperature, as did catecholamines. Smaller doses of clonidine were required than of noradrenaline, although on an equimolar basis, sleep was briefer with clonidine whereas the hypothermic effects at thermoneutrality were longer lasting. Remarkably, in adult fowls, intravenous clonidine induced much more prolonged behavioural and slow wave electrocortical sleep than was obtained with an identical dose given intraventricularly; since clonidine crosses the blood-brain barrier, access to brain areas involved in sleep was clearly superior from the blood than from the cerebrospinal fluid. Sleep following clonidine appeared to be due to an action on central α-adrenoceptors, since it was attenuated by phentolamine but not by intraventricular doses of antagonists at β-adrenoceptors or to 5-hydroxytryptamine, dopamine or acetylcholine. Holman *et al.* (1971) concluded that clonidine evoked sleep in young chicks by an action on central α-adrenoceptors. There is evidence that central α-adrenoceptors, mediating clonidine-induced sleep, differ from those in the periphery (Delbarre & Schmitt, 1973).

The findings with clonidine reinforce the notion that noradrenaline, the neurotransmitter acting on central α-adrenoceptors, is important for sleep associated with slow wave electrocortical activity, despite such emphatic claim that 5-hydroxytryptamine is the amine involved. Indeed,

sleep with slow wave electrocortical activity was induced in fowls both by centrally applied noradrenaline (Grunden & Marley, 1970; Marley & Stephenson, 1970; Marley & Nisticò, 1972) or 5-hydroxytryptamine (Marley & Nisticò, 1975; Marley & Whelan, 1975a), providing grounds for assuming involvement of noradrenergic as well as 5-hydroxytryptaminergic systems. Since clonidine elicited sleep in rats previously rendered awake by *p*-chlorophenylalanine, Holman *et al.* (1971) concluded it was not mediated through the ascending 5-hydroxytryptaminergic system. 'Wakefulness' induced by *p*-chlorophenylalanine is one of the strongest pieces of evidence for implicating 5-hydroxytryptamine in electrocortical slow wave sleep; however, any assumption that this can be equated with normal wakefulness has been vigorously criticized (Rechtschaffen, Lovell, Freedman, Whitehead & Aldrich, 1973), and to complicate interpretation, *p*-chlorophenylalanine has pharmacological actions other than tryptophan hydroxylase inhibition (Marley & Whelan, 1975b).

Despite similarities in sleep provoked by clonidine and noradrenaline, there were differences in their other effects, possibly because of clonidine's greater potency. Thus, activation of heat-loss mechanisms was much more evident with clonidine. For example, and not previously reported in the fowl or other species, clonidine evoked marked tachypnoea (respiratory heat loss) at thermoneutral ambient temperatures; wing abduction and less constantly, increase in comb and leg temperatures developed, all promoting radiant heat loss. These changes were evident before any considerable, i.e. $> 0.5^{\circ}$C, decline of body temperature. Decrease in peripheral vasomotor tone was inferred from the increases in comb and unfeathered leg temperatures after clonidine; however, comb temperature only increased when it was initially low, that is when ambient temperature was at or near the lower end of the thermoneutral range. Once the fall in body temperature with clonidine was maximal, heat-conservation replaced heat-loss mechanisms, viz. feather erection, wing droop and squatting (by squatting, fowls reduced heat loss by one-third compared with standing fowls; Deighton & Hutchinson, 1940).

Heat-loss mechanisms were also activated by relatively large doses of catecholamines. Thus noradrenaline infused into the hypothalamus or IIIrd ventricle induced tachypnoea and wing abduction; intraventricular isoprenaline or dopamine also evoked tachypnoea (Marley & Nisticò, 1972). The fall in body temperature following noradrenaline was accompanied by elevation or depression of comb temperature, according to whether the latter was initially low or high; elevation of comb temperature was not sustained, as after clonidine. Increase in skin temperature of the unfeathered feet follows application of noradrenaline to the hypothalamus of adult pigeons or young chicks (Hissa & Rautenberg, 1974; Marley & Stephenson, 1975). As with clonidine, once the fall in body temperature was marked, heat-conservation replaced heat-loss mechanisms.

Augmented heat-loss could therefore partly account for the fall in body temperature after clonidine. Additionally, CO_2 elimination was 50% reduced, implying decreased metabolism which would also lower body temperature. The decline in CO_2 elimination, as with the heat-loss mechanisms, was well developed before body temperature had fallen to any extent. This decline resembled that induced in chicks below thermoneutrality by noradrenaline infused into the hypothalamus, a decline attributed to inhibition of utilization of non-esterified fatty acids (Marley & Stephenson, 1975); clonidine may well act similarly. Fowls utilize lipids rather than carbohydrates for heat production (Freeman, 1967). The fall in body temperature with clonidine was enhanced and prolonged when the fowl was at an ambient temperature below thermoneutrality, a feature noted with intravenous or intrahypothalamic infusion of catecholamines (Allen & Marley, 1967; Allen, Garg & Marley, 1970; Marley & Stephenson, 1975).

Intraventricular phentolamine attenuated the hypothermic effects of centrally applied clonidine. The dose of phentolamine was crucial, since doses two to four times those used for the antagonism experiments provoked marked and even fatal hyperthermia. Other antagonists administered intraventricularly, viz propranolol, haloperidol, methysergide and atropine, did not attenuate the hypothermic effects of clonidine.

Feldberg & Saxena (1971) tested the effect of α-adrenoceptor antagonists given intraventricularly on the assumption that noradrenaline was released continuously in the hypothalamus to control body temperature. Compatible with this idea, in cats, which respond like fowls with a fall in body temperature to centrally applied noradrenaline, hyperthermia developed after intraventricular phenoxybenzamine or phentolamine (Feldberg & Saxena, 1971). Since noradrenaline was also hypothermic in fowls at or below thermoneutrality, it was scarcely surprising that blockade of its receptors elevated body temperature. Intraventricular atropine also elevated body temperature, a finding more difficult to interpret since acetylcholine and other cholinomimetics infused intraventricularly or into the hypothalamus of chickens at thermoneutrality, lacked temperature

effects (Marley & Seller, 1972, 1974a, b); a hypothermic action of these substances did emerge when chickens were tested below thermoneutrality (Stephenson & Marley, unpublished). It seems therefore that metabolic processes in the fowl maintain a high body temperature, exceeding that in mammals, which is kept within physiological limits at thermoneutrality by hypothalamic release of noradrenaline and possibly of acetylcholine. Since intraventricular methysergide, in doses effective against centrally applied 5-hydroxytryptamine, did not affect body temperature it would appear that 5-hydroxytryptamine release into the fowls' hypothalamus had little to do with temperature regulation at thermoneutrality.

The interaction between clonidine and dexamphetamine was of interest. Thus, behavioural and electrocortical arousal induced by dexamphetamine in adult fowls were replaced by sleep following clonidine infused intraventricularly, into the hypothalamus or intravenously. Similarly, arousal elicited by dexamphetamine was replaced by sleep following catecholamines given intravenously in young chicks (Key & Marley, 1962; Dewhurst & Marley, 1965), and intraventricularly or into the hypothalamus of adult fowls (Marley & Nisticò, 1975). These results, together with the evidence that clonidine and noradrenaline act on central α-adrenoceptors, are difficult to reconcile with the notion that central noradrenaline release mediates arousal, whether drug-induced or not.

There are possible explanations for these findings. First, intraventricular clonidine and noradrenaline may affect different populations of central α-adrenoceptors from those involved in arousal associated with amphetamine-induced noradrenaline release. While this is likely, it still does not explain replacement of dexamphetamine-induced arousal by sleep following intravenous noradrenaline in young chicks or intravenous clonidine in young or adult fowls, since the overall brain distributions of the three compounds after administration were likely to be similar.

Clonidine and exogenous noradrenaline may have different effects from noradrenaline liberated endogenously. The evidence is against this for certain parts of the brain, since the soporific and hypothermic effects of noradrenaline infused into the IIIrd cerebral ventricle or hypothalamus were markedly potentiated by amine oxidase inhibition (Schain, 1961; Schmidt & Fähse, 1964; Marley & Stephenson, 1970; Marley & Nisticò, 1972), potentiation implying that noradrenaline was taken up into neurones, escaped deamination and was then released, i.e. endogenously. Moreover, dexamphetamine given intraventricularly to cats (Gaddum & Vogt, 1956) or infused into the hypothalamus of fowls (Marley & Nisticò, 1975) induced sleep, that is noradrenaline released endogenously by dexamphetamine, mimicked the effects of exogenous noradrenaline.

Extending this argument to other parts of the brain, it could be that the excitant action of dexamphetamine was due to the effects of noradrenaline released in the brain-stem preponderating over those of noradrenaline liberated elsewhere in the brain, particularly the hypothalamus (Marley, 1973). This is feasible, since noradrenaline perfused bilaterally into the pontine and mesencephalic reticular formation of feline encéphale isolé preparations, evoked behavioural and electrocortical arousal (Key, 1975), whereas noradrenaline given intraventricularly or into the hypothalamus induced sleep (Feldberg & Sherwood, 1954; Myers, 1964).

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