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Effects of catecholamines and adenosine derivatives given into the brain of fowls

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Summary

1. Adult fowls (*Gallus domesticus*) with cannulae chronically implanted into the IIIrd cerebral ventricle and various other sites of the brain received micro-infusions or injections of catecholamines, adenosine, 3′,5′-cyclic AMP or its dibutyryl derivative. The effects of these substances on behaviour, electrocortical activity and body temperature were studied.

2. Behavioural and electrocortical sleep with fall in body temperature were obtained with intraventricular noradrenaline, α-methylnoradrenaline and isoprenaline; dopamine was ineffective. The doses required to elicit sleep were smaller than those affecting body temperature. Following mebanazine, the effects of noradrenaline were prolonged and doses of dopamine, previously ineffective, lowered body temperature and induced behavioural and electrocortical sleep.

3. Noradrenaline, α-methylnoradrenaline, isoprenaline and dopamine infused into the hypothalamus induced sleep and lowered body temperature. Effective doses of noradrenaline, α-methylnoradrenaline and isoprenaline infused into the hypothalamus were one-twentieth to one-fifth those for intraventricular injection. Tachypnoea developed with isoprenaline and dopamine. Additionally with dopamine, there was deviation of the head to the contralateral side, together with repetitive jerking movements of the head. These effects were prolonged and intensified by mebanazine, whereas the involuntary movements with dopamine were greatly reduced by haloperidol.

4. Involuntary movements, but without sleep, were induced by infusing dopamine into the paleostriatum augmentatum; noradrenaline infused into this site was ineffective.

5. In three of five fowls pretreated with aminophylline, 3′,5′-cyclic AMP infused into the hypothalamus induced behavioural and electrocortical sleep; without aminophylline pretreatment, 3′,5′-cyclic AMP was ineffective. Adenosine infused into the hypothalamus, following pretreatment of fowls with aminophylline, consistently induced behavioural and electrocortical sleep. Dibutyryl cyclic AMP infused into the hypothalamus of intact fowls elicited behavioural arousal, followed by bursts of electrocortical spikes (6 Hz) over both cerebral hemispheres, spikes subsequently becoming regular at 1 Hz. Clonic limb and body movements occasionally accompanied the bursts of spike activity, infrequently developing into convulsions. In fowl *encéphale isolé* preparations, in which dibutyryl cyclic AMP was infused into the hypothalamus, spike activity was confined to the ipsilateral hemisphere.

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Introduction

Catecholamines infused into the hypothalamus of young chicks (Marley & Stephenson, 1970) or injected into the IIIrd cerebral ventricle of adult fowls (Grunden & Marley, 1970) elicited behavioural and electrocortical sleep and lowered body temperature. The present study was undertaken to compare potency and effects of catecholamines given by both routes in the same animals; since chronic implantation experiments with intracerebral cannulae are vitiated in young chicks by rapid skull growth, experiments were performed in adult fowls.

Experiments were also performed to ascertain whether adenosine 3′,5′-cyclic monophosphate (3′,5′-cyclic AMP) simulated the central effects of catecholamines; its actions were compared with those of adenosine and a longer-lasting congener, dibutyryl adenosine 3′,5′-cyclic monophosphate (dibutyryl cyclic AMP).

A brief account of this work has been published (Marley & Nistico, 1971).

Methods

Operative procedures

Rhode Island Red hens weighing 1·5 to 2·0 kg were used. Cannulae, electrodes and thermistors were implanted under aseptic conditions, the chickens being anaesthetized with halothane (Marley & Payne, 1964). In some chickens, one cannula was implanted stereotactically in the hypothalamus and in others two cannulae were implanted, one in the hypothalamus and the other in the IIIrd cerebral ventricle. Guide and injection cannulae together with stylets were prepared as described by Marley & Stephenson (1968). Guide cannulae were implanted using the following co-ordinates (van Tienhoven & Juhász, 1962): right hypothalamus A+7·5, L=1, H+4·0; IIIrd ventricle, A+6·5, L=0, H+5·5; and at various sites in the paleostriatum augmentatum [nucleus basalis, nomenclature of Kuhlenbeck (1938) as used by Juorio & Vogt (1967)] and in the mesencephalon. Correct placement of ventricular cannulae was established by outflow of clear cerebrospinal fluid at implantation and for both cannulae by subsequent histological examination of the brain.

The preparation and implantation of cortical recording electrodes and insertion of a thermistor in the interscapular subcutaneous tissue were as previously described (Allen & Marley, 1967; Marley & Key, 1963). The electrode wires and thermistor leads were soldered to separate contacts of a 10-pin miniature electrical socket (Ether Ltd.) and the entire assembly fixed to the skull with acrylic dental cement (Dental Fillings Ltd.).

For *encéphale isolé* preparations, in which cannulae and electrodes had been implanted at least one day previously and a hole drilled in the cranium to expose the left side of the cerebellum, the fowl was reanaesthetized with halothane, a blunt leucotome was inserted through the cerebellum, and gentle pressure exerted to transect the brain stem immediately above its junction with the spinal cord. Anaesthesia was stopped and artificial respiration continued. To ensure satisfactory recovery after halothane, at least 30 min elapsed before recording began. The completeness of brain section was ascertained at the end of the experiment.

Experimental procedures

Apart from the *encéphale isolé* preparations, fowls were not tested until at least a week after operation and thereafter at weekly intervals. Experiments were

carried out with the fowl in a wooden recording chamber having a one-way observation window in the door. The chamber was illuminated with 12 V d.c. bulbs and housed in a sound-insulated room. Air temperature within the chamber was maintained between 20° and 25° C, i.e., within the thermoneutral range for adult fowls (Barott & Pringle, 1946). A 9-conductor shielded cable provided electrical connexion between the animal and recording instruments. Bipolar recordings of electrocortical activity were made on a Kaiser 8-channel electroencephalograph and electrocortical activity integrated at minute intervals by a modification of the method of Dewhurst & Marley (1965). Temperature was recorded on a thermistor thermometer (Grant Instruments Ltd.).

Intracerebral and intraventricular injections were made with an injection cannula attached by a length of polyethylene tubing to a Hamilton gas-tight 10 μl syringe. Volumes of 1 to 2 μl were infused into the brain and 4 to 6 μl injected into the IIIrd ventricle, at a rate of 1 μl/minute. For intracerebral infusions, the polyethylene tubing passed from the fowl, through the roof of the chamber and was connected externally to the syringe driven by a slow-infusion pump. Control infusions of 0·9% NaCl w/v (saline) lacked effect on behaviour and electrocortical activity; body temperature changed up to $\pm$0·5° C, briefly.

Drugs

These (with the molecular weight of the salts in parentheses) were the hydrochlorides of dopamine (190), (−)-α-methylnoradrenaline (220) and ($\pm$)-α-methyltryptamine (210); the sulphates of (−)-isoprenaline (557) and dexamphetamine (368); the sodium salt of N^6,O^2-dibutyryl adenosine 3′,5′-cyclic-mono-phosphoric acid (491) and mebanazine oxalate (226). Other drugs used were adenosine (267), adenosine 3′,5′-cyclic-mono-phosphoric acid (347), aminophylline (258, theophylline ethylenediamine), pimozide (461) and haloperidol (376). (−)-Noradrenaline base (169) was dissolved immediately before use in equimolar hydrochloric acid.

Results

Catecholamines

(−)-Noradrenaline

Injection into the IIIrd ventricle. Noradrenaline, 0·5 μmol, given intraventricularly in 8 fowls elicited behavioural and electrocortical sleep and lowered body temperature by 1·5° C. Sleep commenced 1 to 5 min after injection and lasted 2 to 3 h, the fowl squatting or standing in a ' tripod ' position with the head lowered and the beak resting on the ground; noradrenaline, 0·25 μmol, was ineffective.

Infusion into the hypothalamus. Similar effects were produced by infusing one-fifth of the intraventricular dose (0·1 μmol), into the hypothalamus of 15 fowls except that electrocortical sleep activity was of larger amplitude after intraventricular injections (Fig. 1, B and D); noradrenaline, 0·025 μmol, infused into the hypothalamus elicited behavioural sleep lasting 2 h, but did not affect body temperature.

Infusion into other brain areas. Noradrenaline, 0·075 to 0·2 μmol, infused into the mesencephalon, paleostriatum augmentatum and right telencephalon was without effect.

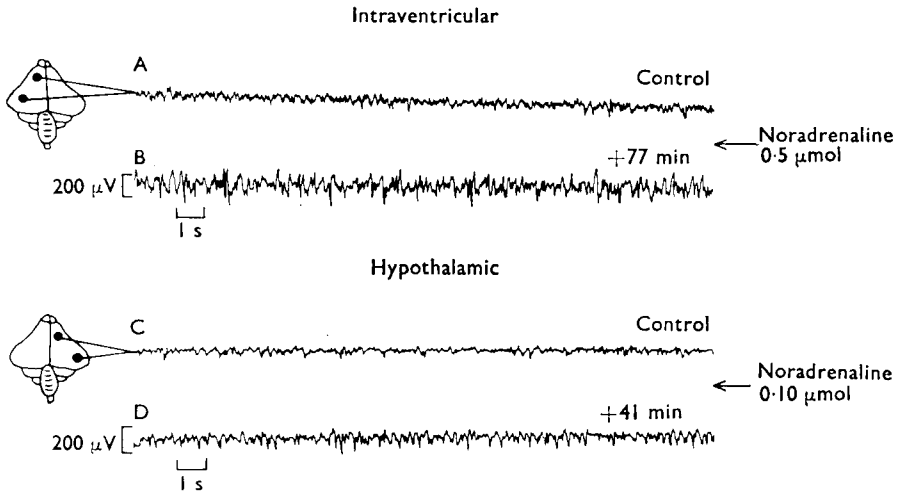

FIG. 1. Electrocortical activity from 2 adult fowls. A, C, Control alert electrocortical potentials. B, Large amplitude (100–400 μV), 5–8 Hz sleep electrocortical potentials 77 min after intraventricular noradrenaline. D, Smaller amplitude (60–140 μV), 6–8 Hz typical sleep potentials 41 min after intrahypothalamic infusion of noradrenaline.

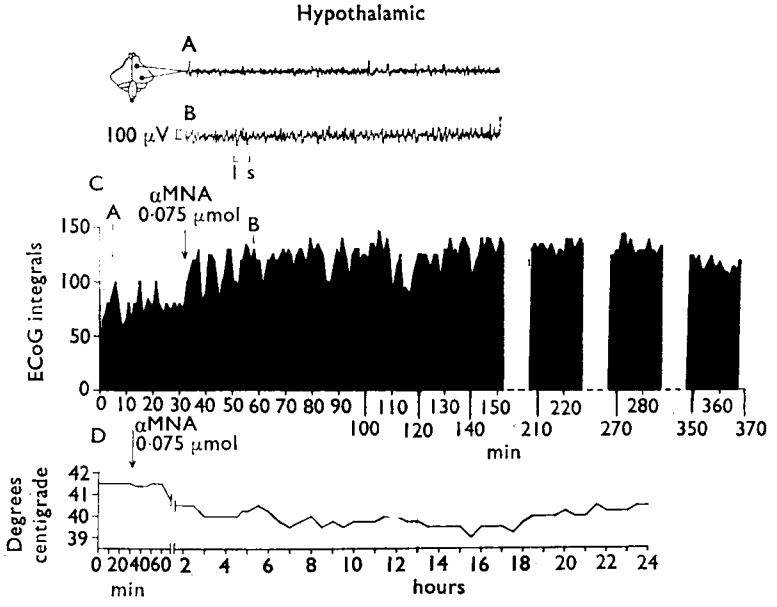

FIG. 2. Effects of α-methylnoradrenaline (αMNA) infused into the hypothalamus of an adult fowl. From above downwards, traces of electrocortical activity (A, B), histograms of integrated electrocortical activity (C) and graph of body temperature (D). A, Alert control electrocortical activity; B, electrocorticogram during sleep induced by α-methylnoradrenaline (0·075 μmol). C, Integrals corresponding to electrocortical activity and showing sustained increase for up to 5·5 h after α-methylnoradrenaline. D, Fall of 2·5° C in body temperature with incomplete recovery 24·5 h after α-methylnoradrenaline.

(−)-α-Methylnoradrenaline

Infusion into the hypothalamus. α-Methylnoradrenaline, 0·01 to 0·2 μmol, infused into the hypothalamus of 19 fowls produced similar behavioural and electrocortical effects to those evoked by noradrenaline, except that the duration of action was longer than 6 h and occasionally greater than 24 hours. Thus following α-methylnoradrenaline, electrocortical activity changed from a low amplitude, fast frequency alert pattern (Fig. 2A) to a large amplitude, slow frequency sleep pattern (Fig. 2B) associated with increase of electrocortical activity integrals (Fig. 2C); occasionally, low amplitude 'paradoxical' sleep patterns appeared during α-methylnoradrenaline-induced sleep. Body temperature, which began to fall 30 min after the infusion, was lowered by a maximum of 2·5° C (Fig. 2D); recovery was complete at 36 hours.

Infusion into other brain areas. α-Methylnoradrenaline, 0·05 to 0·2 μmol, infused into the mesencephalon and right telencephalon was without effect (3 fowls).

Injection into IIIrd ventricle. To induce effects similar to those obtained with intrahypothalamic α-methylnoradrenaline, intraventricular doses at least five times larger were required (0·5 to 1·0 μmol).

(−)-Isoprenaline

Infusion into the hypothalamus. Isoprenaline (0·025–0·2 μmol) infused into the hypothalamus of eight fowls produced behavioural and electrocortical sleep lasting 3 to 6 h, accompanied by postural changes as described with the other catecholamines; its duration of action was intermediate between that of equimolar doses of noradrenaline and α-methylnoradrenaline. As shown in Fig. 3, following intrahypothalamic infusion of isoprenaline (0·1 μmol), the alert electrocortical pattern (Fig. 3A) changed to that of sleep (Fig. 3B); electrocortical activity integrals rose to a peak which was four times that of the control period and temperature declined 1·25° C (Fig. 3D). Within 5 min of infusion, respiratory rates rose from a control of 20–30/min to about 120/min lasting 15 to 30 minutes.

Injection into IIIrd ventricle. Isoprenaline, 0·1 μmol, given intraventricularly to the same eight fowls was without apparent effect, whereas 0·5 μmol elicited similar effects to those of 0·1 μmol infused into the hypothalamus.

Dopamine

Infusion into the hypothalamus. Dopamine (0·1 μmol) infused into the hypothalamus induced sleep for 30 min in two of five fowls tested. In another fowl, dopamine (0·4 μmol) produced behavioural and electrocortical sleep for 1 h, temperature falling 1° C with recovery after 3 hours. Tachypnoea (40–60/min) developed after 15 min and abated at about 1 hour. In two of the fowls, 15–20 min after intrahypothalamic infusion of dopamine (0·1 and 0·4 μmol respectively), deviation of the head to the side contralateral to that of the infusion was observed together with repetitive jerking movements of the head and neck from this position towards the midline. These phenomena persisted for 30 to 45 minutes.

Injection into IIIrd ventricle. When injected into the IIIrd ventricle, dopamine, 0·5 μmol, was without effect.

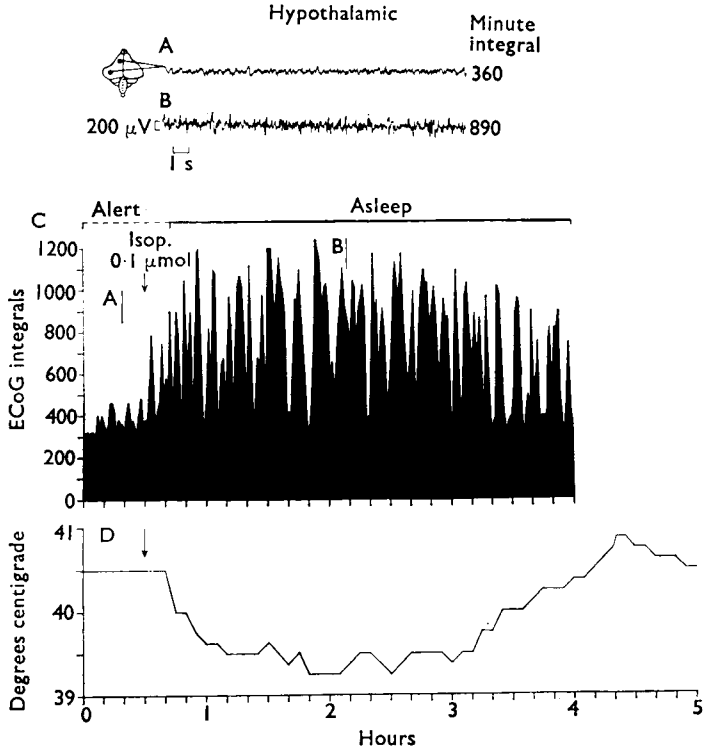

FIG. 3. Effects in an adult fowl of isoprenaline (Isop) infused into the hypothalamus. From above downwards, traces of electrocortical activity (A, B), histogram of integrated electrocortical activity (C) and graph of body temperature (D). A, Alert control electrocortical activity. B, Electrocorticogram during sleep induced by isoprenaline (0·1 μmol). C, Integrals corresponding to electrocortical activity and showing fluctuating increase for up to 3·5 h after isoprenaline. D, Fall in body temperature of 1·25° C with recovery 220 min after isoprenaline, followed by a rise in temperature above pre-injection values.

Catecholamines and monoamine oxidase inhibitors

Noradrenaline and dopamine are metabolized within neurones by monoamine oxidase. These amines were therefore tested after a monoamine oxidase inhibitor, mebanazine, to ascertain whether their effects were enhanced.

(−)-Noradrenaline

Injection into IIIrd ventricle. After pretreatment of 2 fowls with mebanazine (100 μmol/kg i.p. 18 and 1 h previously), an intraventricular dose of noradrenaline 0·25 μmol, that had previously been ineffective, produced behavioural and electrocortical sleep lasting over 6 hours. The effects of noradrenaline (0·5 μmol), which itself induced sleep and lowered body temperature, were significantly enhanced by pretreatment of 2 fowls with the above dose of mebanazine.

Infusion into the hypothalamus. The effect of mebanazine on noradrenaline infused into the hypothalamus was investigated in 3 fowls; data from one of these are shown in Figure 4. A control infusion of noradrenaline (0·025 μmol) into the hypothalamus produced behavioural and electrocortical sleep (Fig. 4B), lasting 3 hours. Integrated electrocortical activity increased during sleep and returned to pre-injection values on recovery (Fig. 4D). Body temperature fell

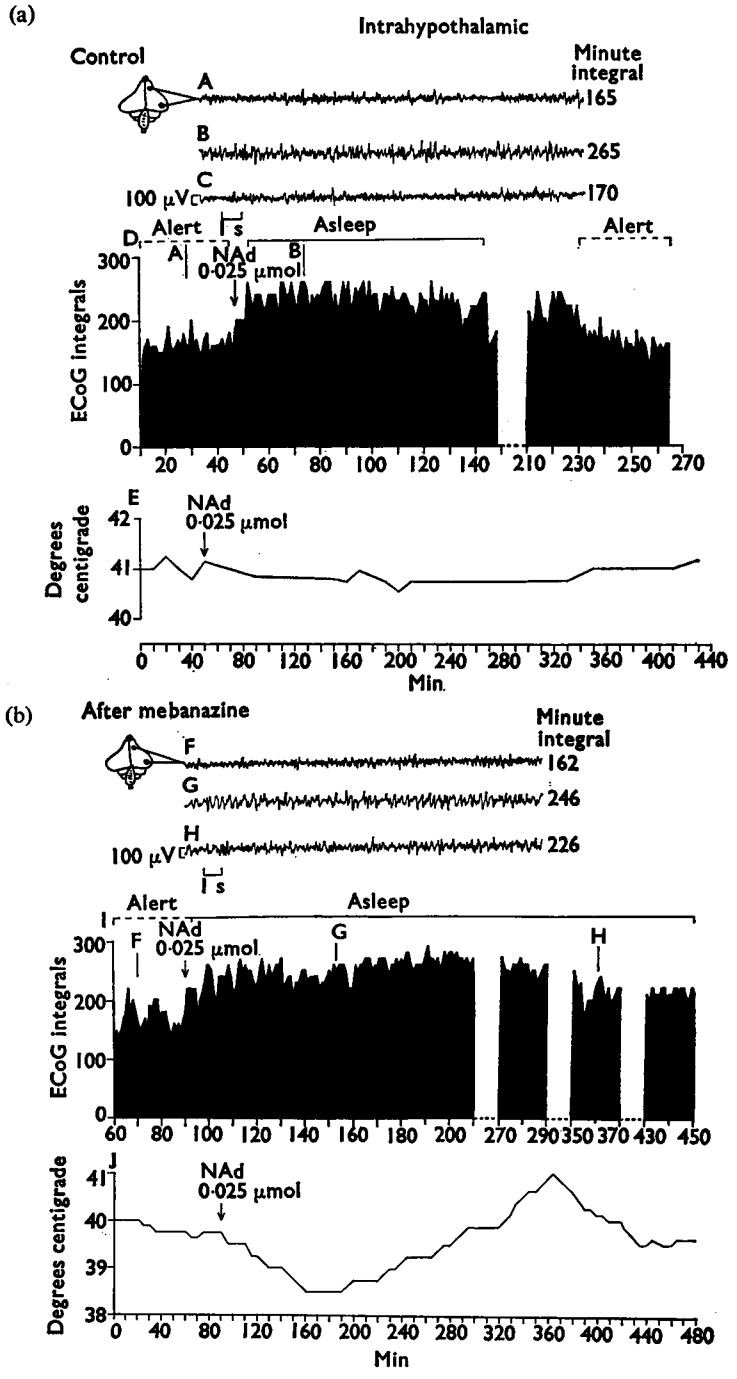

FIG. 4. Effects of noradrenaline (0·025 μmol) infused into the hypothalamus of an adult fowl. A to E, Control experiment; F to H, following pretreatment with mebanazine. *Control experiment.* A, Alert electrocortical pattern before noradrenaline; C, alert pattern on recovery 190 min after noradrenaline. B, Electrocortical potentials during sleep induced by noradrenaline. D, Integrals corresponding to electrocortical activity and showing increase for 180 min after noradrenaline. E, No significant effect of noradrenaline on body temperature. *After mebanazine.* F, Alert electrocortical pattern before noradrenaline. G, H, Electrocortical sleep potentials 85 and 290 min respectively after noradrenaline. I, Integrals corresponding to electrocortical activity and showing sustained increase for up to 380 min after noradrenaline. J, Fall in body temperature of 2° C with recovery after 200 min, followed by a 1·25° C increase above pre-injection values.

0·45° C from 41° C (Fig. 4E). The fowl was then rested for 5 days. After mebanazine (100 μmol/kg i.p. 18 and 1 h previously), noradrenaline (0·025 μmol) elicited electrocortical (Fig. 4G, H) and behavioural sleep persisting for more than 8 h ; there was an associated increase in electrocortical activity integrals. Temperature fell 2° C from 39·75° C, with recovery after 200 min (Fig. 4J) ; thereafter, temperature rose to 41° C, 1·25° C above preinjection values. Thus mebanazine pretreatment prolonged the soporific effects of noradrenaline at least three-fold and intensified its hypothermic action.

Dopamine

Injection into IIIrd ventricle. Intraventricular dopamine (0·5 μmol), ineffective on its own, produced profound behavioural and electrocortical sleep lasting about 3 h in 2 fowls pretreated with mebanazine (100 μmol/kg i.p. 18 h and 1 h previously), body temperature falling 0·75° C and 1·75° C respectively with recovery after 4 hours. Vertical and horizontal jerking movements of the head and neck developed after 15 min, and these effects persisted for 60 minutes.

Infusion into the hypothalamus. Intrahypothalamic dopamine, after mebanazine, had similar effects to intraventricular dopamine after mebanazine, although effective doses were smaller. Thus dopamine (0·1 or 0·15 μmol) infused into the hypothalamus of four fowls, after mebanazine (100 μmol/kg i.p. 18 h and 1 h previously), immediately produced sleep which lasted for more than 3 hours. Body temperature of the 4 fowls fell by 0·75° to 2·0° C after dopamine. In three, this fall was immediate, suggesting the effect was due to dopamine rather than to noradrenaline displaced from stores by dopamine ; in the other, results from which are plotted in Fig. 5B, dopamine (0·15 μmol) infused into the hypothalamus evoked not only an immediate fall in temperature (max 0·75° C) with return to preinjection values after 85 min, but also a delayed, larger fall (max 1·75° C) which lasted for more than 3 hours. The possibility of the later fall of temperature, starting 2 h after dopamine, being due to release of noradrenaline newly synthesized from dopamine cannot be discounted. In rats, the maximal concentration of noradrenaline in the hypothalamus was found 30 min after intraventricular injection of dopamine (Glowinski & Iversen, 1966). In the control test with this fowl prior to mebanazine, dopamine (0·15 μmol) infused into the hypothalamus had little effect on temperature (Fig. 5A) and 0·4 μmol, a smaller effect than 0·1 μmol after mebanazine. Dopamine, infused into the hypothalamus or injected intraventricularly, induced tachypnoea (50–80/min), developing 10 to 15 min after injection and persisting for about 1 hour.

Deviation of the head to the contralateral side together with jerking movements of the head were observed in 3 of these 4 fowls infused with dopamine. These effects were quantitated in a further 4 fowls similarly pretreated with mebanazine. Dopamine (0·2 μmol) was infused into the hypothalamus and once the 'involuntary' head movements developed, these were counted for two consecutive 30 min periods (first test), starting 20 min after dopamine infusion, a time when the phenomena were well established. A week later the procedure was repeated (second test).

The effect of haloperidol on these movements was investigated. Haloperidol is a selective dopamine antagonist at peripheral receptors (van Rossum, 1966 ; Yeh,

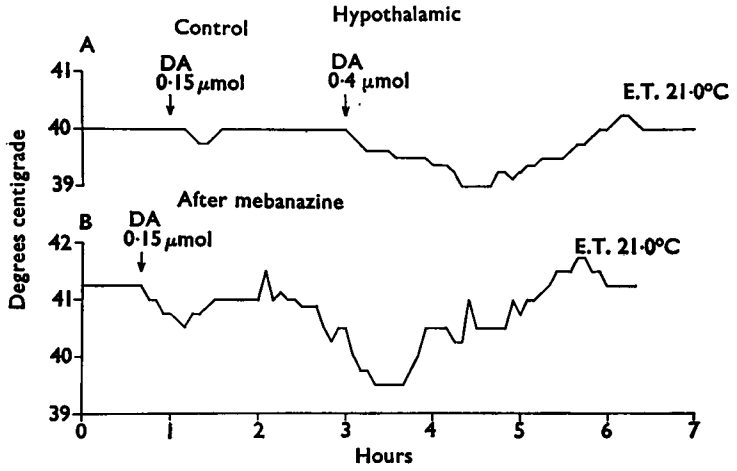

FIG. 5. Effects on body temperature of dopamine infused into the hypothalamus of an adult fowl in two experiments at an environmental temperature (E.T.) of 21° C. Dopamine (DA), 0·15 μmol, after mebanazine and a more profound and longer-lasting effect on body temperature (B) than dopamine, 0·4 μmol (A), in the same fowl but without mebanazine pretreatment.

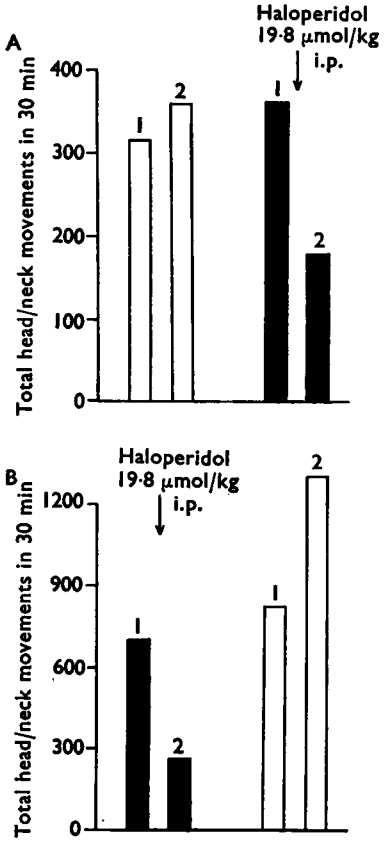

FIG. 6. Histograms of mean number of head/neck movements in two consecutive periods (1, 2) of 30 min, starting 20 min after dopamine infusion into the hypothalamus in fowls pretreated with mebanazine. In A, mean counts from two fowls in which haloperidol was given in the second of the experiments (solid histograms), one week following the first (open histograms); in B, the haloperidol experiments (solid histograms) preceded by one week those without haloperidol (open histograms). Haloperidol substantially reduced the number of head/neck movements.

McNay & Goldberg, 1969), although evidence for antagonism in the central nervous system is circumstantial. For 2 of the 4 fowls, haloperidol (19·8 μmol/kg i.p.) was given in the first of the two tests immediately after the initial 30 min count (Fig. 6B) and for the other two, in the last of the two tests (Fig. 6A). Following haloperidol, the count was reduced 50% (Fig. 6A) and 63% (Fig. 6B) respectively, compared to the control experiments in which the counts during the second 30 min exceeded those of the first. Two further fowls given dopamine after mebanazine, were tested to ensure that saline 1·5 ml/kg i.p. (the same volume as for haloperidol) injected between the first and second of the 30 min counts, did not reduce the latter. In both fowls, the mean count for the second period (613·5 head/neck movements) exceeded that for the first (438 head/neck movements), as in the control experiments without saline. In another fowl, movements evoked by dopamine were reduced by pimozide (16·8 μmol/kg i.p.)—also a dopamine

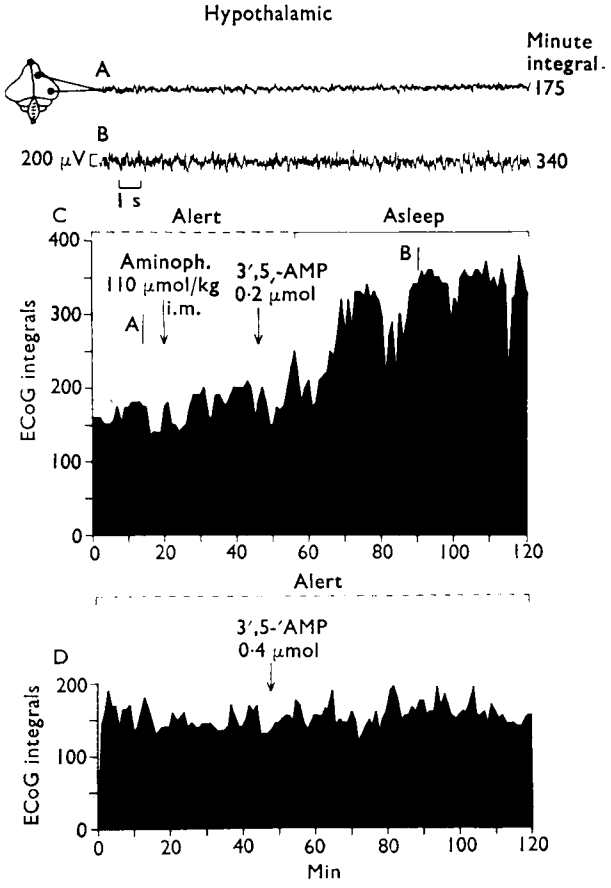

FIG. 7. Effects in an adult fowl of cyclic AMP (3′,5′-AMP) infused into the hypothalamus following intramuscular aminophylline. From above downwards, traces of electrocortical activity (A, B) and histograms of integrated electrocortical activity (C, D). A, Alert electrocortical activity. B, Electrocorticogram during sleep induced by cyclic AMP, 0·2 μmol, following aminophylline. C, Integrals corresponding to electrocortical activity and showing sustained increase during sleep. D, Electrocortical integrals from the same fowl. Double the dose (0·4 μmol) of cyclic AMP, but no treatment with aminophylline, was without effect on behaviour and did not alter alert electrocortical activity or integrals.

antagonist, but after a longer delay than with haloperidol. Neither haloperidol nor pimozide attenuated sleep induced by dopamine.

Infusion into the paleostriatum augmentatum. The paleostriatum augmentatum lies immediately anterior to the hypothalamus and contains a large concentration of dopamine (Juorio & Vogt, 1967). The 7 fowls tested were pretreated with mebanazine (100 μmol/kg i.p. 18 and 1 h previously), and dopamine (0·2 or 0·3 μmol) was then infused. Involuntary movements and postural abnormalities developed in all seven birds, similar to but less intense than those after intrahypothalamic infusion of dopamine alone (2 of 5 fowls) or with dopamine after mebanazine (7 of 8 fowls); the delay before onset and the duration of these effects were also similar to those following intrahypothalamic dopamine infusions. In contrast to infusions into the hypothalamus, profound sleep did not develop, 5 of the fowls remaining alert throughout the experiment and two becoming mildly drowsy. Respiratory rate was elevated (60–80 min).

3′,5′-cyclic AMP, adenosine and dibutyryl cyclic AMP

3′,5′-cyclic AMP

Since 3′,5′-cyclic AMP given intraventricularly (0·3 and 0·4 μmol) or infused into the hypothalamus (0·1, 0·2 and 0·4 μmol) of five fowls was without effect on behaviour, electrocortical activity or body temperature, its effects were examined after administering aminophylline, a phosphodiesterase inhibitor (Butcher & Sutherland, 1962; Siggins, Hoffer & Bloom, 1971). In preliminary tests, aminophylline (110 μmol/kg i.m.) was found to have no effect in fowls on behaviour or temperature; the same dose of theophylline, given to rats, potentiates the metabolic effects of 3′,5′-cyclic AMP (Brodie, Davies, Hynie, Krishna & Weiss, 1966).

Infusion into the hypothalamus. Of 5 fowls in which 3′,5′-cyclic AMP (0·1, 0·2 or 0·3 μmol) was infused into the hypothalamus, 25 to 50 min after aminophylline (110 μmol/kg i.m.), electrocortical and behavioural sleep lasting 2 to 2·5 h developed in three. The development of electrocortical sleep, together with associated increase in electrocortical activity integrals following infusion of 3′,5′-cyclic AMP, 0·2 μmol, in a fowl pretreated with aminophylline (110 μmol/kg i.m.) is shown in Fig. 7B, C. Double the dose of 3′,5′-cyclic AMP (0·4 μmol), infused into the same fowl but without aminophylline pretreatment, was ineffective (Fig. 7D).

Adenosine

Sleep was not consistently obtained with 3′,5′-cyclic AMP, possibly due to its poor penetration. However, adenosine—which has a specific uptake mechanism into cells, caused a 30- to 40-fold increase in 3′,5′-cyclic AMP when added to isolated cerebral tissues (McIlwain, 1971).

Infusion into the hypothalamus. Adenosine, 0·1 to 0·3 μmol, infused into the hypothalamus induced sleep lasting 10 to 30 min, but again its effect was inconsistent. However, adenosine, 0·1, 0·15 and 0·2 μmol infused 30 to 45 min after aminophylline produced immediate behavioural and electrocortical sleep lasting 3 to 4 h in all 6 fowls tested. The changes were similar to those with catecholamines i.e. the fowl squatting or standing asleep with bowed head, except that the wings were not lowered—a regular finding with catecholamines. The develop-

ment of electrocortical sleep, with associated increase of electrocortical activity integrals following aminophylline and adenosine given to a previously alert fowl, is shown in Fig. 8. Body temperature changes, although greater than with 3′,5′-cyclic AMP, were not considered significant.

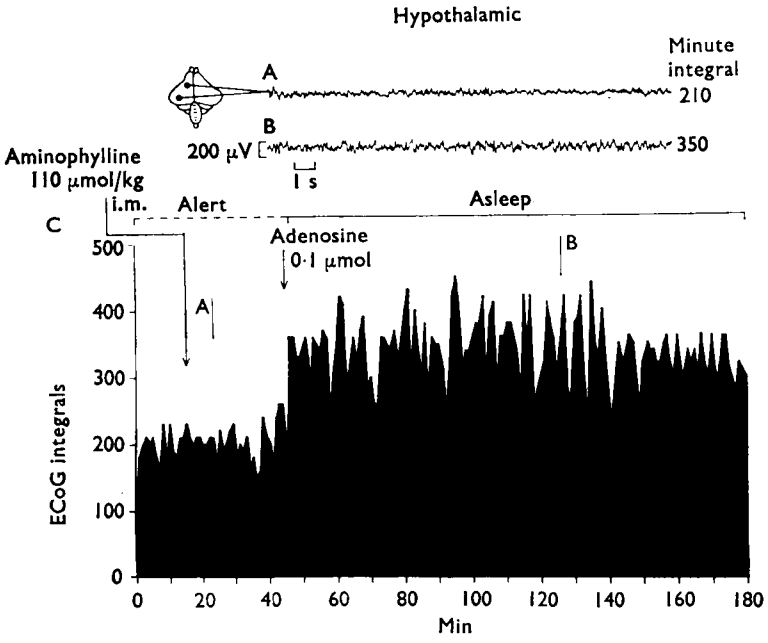

FIG. 8. Effects in an adult fowl of adenosine infused into the hypothalamus following intramuscular aminophylline. From above downwards, traces of electrocortical activity (A, B) and histogram of integrated electrocortical activity (C). A, Alert electrocortical activity. B, Electrocorticogram during sleep induced by adenosine, 0·1 μmol, following aminophylline. C, Integrals corresponding to electrocortical activity and showing sustained increase during sleep.

Injection into IIIrd ventricle. Adenosine, 0·75 and 1·0 μmol respectively, given to two fowls injected with aminophylline (110 μmol/kg i.m. 45 min previously), evoked immediate behavioural and electrocortical sleep lasting 3 to 4 hours.

Dibutyryl cyclic AMP

Infusion into the hypothalamus: intact fowls. Dibutyryl cyclic AMP (0·1 and 0·2 μmol) infused into the hypothalamus of 6 fowls produced a striking but dissimilar picture to that induced by 3′,5′-cyclic AMP or adenosine. Within 5 min of infusion, the fowl was more alert and vocalizing, motor activity being enhanced and culminating in periodic 'escape responses'. Respiratory rate increased to 90-120/minute. Deviation of the head to the contralateral side developed within 2 to 7 min of infusion but was not so sustained as with dopamine, although the vertical and horizontal jerking movements of the head and neck were of greater excursion; rotary movements of the head were also observed. Body temperature rose 0·5° C within 30 min of infusion and by 4 h was as much as 2·25° C above control temperature.

Bilateral bursts of electrocortical spike activity (about 6 Hz) each lasting some

20 s, developed after dibutyryl cyclic AMP (0·2 μmol) as illustrated in Fig. 9B. These bursts recurred intermittently throughout the experiment e.g. at 3, 17 and 34 min after infusion, and were occasionally accompanied by clonic movements of the limbs. Apart from the short bursts of spikes, the electrocorticogram was of an intensely alert pattern during the first 17 min, particularly immediately after the bursts of 6 Hz spike activity; the duration of the alert pattern (Fig. 9C) succeeding the 'spike bursts', dwindled as the experiment progressed. Seventeen min after infusing dibutyryl cyclic AMP, regular spikes of 1 Hz were recorded from both cerebral hemispheres similar to those in Fig. 9D, but of smaller amplitude (200 μV). These were periodically interrupted by large amplitude potentials resembling those during sleep but of regular frequency, or by the bursts of 6 Hz spike potentials. Fifty min after infusion, the electrocorticogram displayed regular spike activity as in Fig. 9D. The behavioural and electrocortical effects lasted for at least 8 hours. Of 6 fowls tested, four had recovered by 24 h but the other two died.

Injection into IIIrd ventricle: intact fowl. Bilateral electrocortical changes were also induced by intraventricular dibutyryl cyclic AMP (1·0 μmol) in the one fowl tested. Spike activity (1 Hz) developed in one cerebral hemisphere 16 min after injection and in the other after 23 minutes.

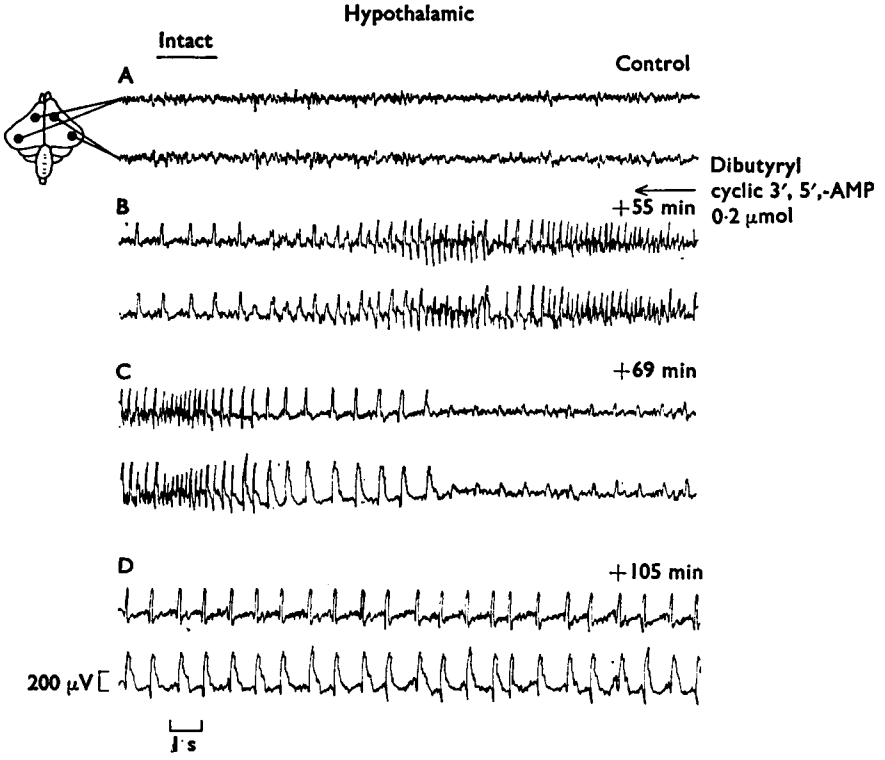

FIG. 9. Electrocortical effects of dibutyryl cyclic AMP (0·2 μmol) infused into the hypothalamus of an adult fowl. A, Alert control electrocortical activity. B, Bilateral 1 Hz electrocortical spike activity 55 min after dibutyryl cyclic AMP, changing to a burst of 6–8 Hz spike potentials. C, Termination of a burst of such potentials, slowing to 1 Hz spikes and followed by 'alert' electrocortical activity with small 1 Hz spike potentials. D, Regular 1 Hz large amplitude spike potentials present over both cerebral hemispheres 105 min after dibutyryl cyclic AMP.

Infusion into the hypothalamus : encéphale isolé *preparations.* Unilateral spike activity (1 Hz) similar to that observed in intact fowls was induced over the ipsilateral hemisphere by intrahypothalamic infusion of dibutyryl cyclic AMP in two fowl *encéphale isolé* preparations ; there was a 10 min delay before its appearance after 0·1 μmol but it developed immediately after 0·4 μmol. With the 0·1 μmol dose, electrocortical activity between spikes was reduced in amplitude in comparison to control activity (Fig. 10B), a reduction more marked with the 0·4 μmol dose and combined with electrocortical arousal over the contralateral hemisphere.

Fowl *encéphale isolé* preparations exhibit persistent behavioural and electrocortical sleep (Marley & Stephenson, 1971), unless arousal is evoked by drugs. Tests were therefore made in two *encéphale isolé* preparations in which arousal had been so induced. In the first, infusion of dibutyryl cyclic AMP (0·2 μmol) into the hypothalamus, after arousal evoked by dexamphetamine (10 μmol/kg i.v.), was immediately followed by the appearance over the ipsilateral hemisphere of spikes of irregular frequency (c.a. 40/min), becoming regular (1 Hz) 15 min after infusion. In the other fowl *encéphale isolé* preparation, with an alert electrocortical activity pattern 55 min after infusion of α-methyltryptamine (0·5 μmol) into the hypothalamus, ' spike and wave ' potentials developed immediately after infusion of dibutyryl cyclic AMP (0·3 μmol) into the hypothalamus of the same side (Fig. 10D).

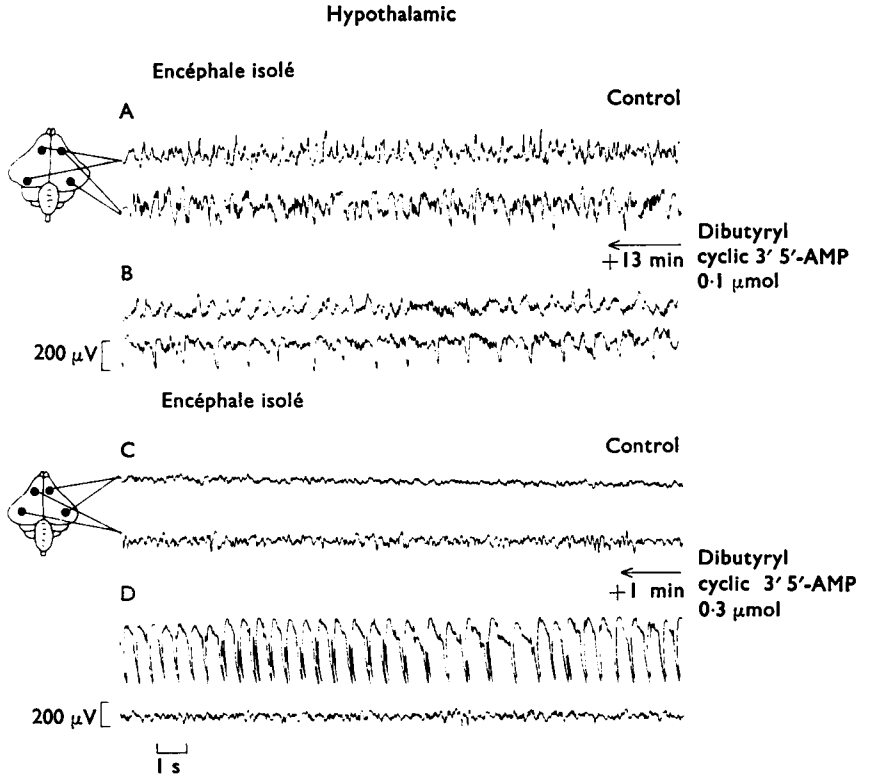

FIG. 10. Electrocortical records from 2 adult fowl *encéphale isolé* preparations (A, B and C, D respectively). A, Control sleep electrocortical activity. B, Regular 1 Hz spike activity confined to right cerebral hemisphere, 13 min after dibutyryl cyclic AMP infused into the ipsilateral hypothalamus. C, Bilateral alert electrocortical activity following α-methyltryptamine (0·5 μmol) infused into the hypothalamus. D, ' Spike and wave ' 2 Hz potentials confined to the right cerebral hemisphere, 1 min after dibutyryl cyclic AMP infused into the ipsilateral hypothalamus.

Discussion

Catecholamines infused into the hypothalamus of young chicks, elicit central depressant effects similar to those obtained on intravenous injection (Marley & Stephenson, 1970). In the present experiments the effects of catecholamines given intraventricularly were found to resemble those induced by intrahypothalamic infusion of the amines in the *same* fowls, inasmuch as behavioural and electrocortical sleep developed and body temperature fell. In the case of infusions, which entail application of catecholamines to a circumscribed brain area, these phenomena were elicited solely from the hypothalamus and no other regions of the brain tested.

Effective doses of catecholamines given intraventricularly were larger than those for infusion into the hypothalamus. Whereas, noradrenaline, 0·25 μmol, injected into the IIIrd ventricle was without effect on behaviour and electrocortical activity, one-tenth the dose (0·025 μmol) infused into the hypothalamus produced behavioural and electrocortical sleep lasting 2 hours. However, electrocortical activity associated with sleep induced by intraventricular injection of catecholamines was invariably of greater amplitude than that evoked by their infusion into the hypothalamus, despite sleep being more profound in the latter. The above findings suggest that brain mechanisms producing large amplitude slow wave electrocortical activity lie outside the hypothalamus or that electrocortical activity following hypothalamic infusions is the 'paradoxical' activity of deep sleep.

Lowering of body temperature by catecholamines infused into the hypothalamus is greater in young than in adult fowls, presumably because of their immature thermoregulatory mechanisms and poor thermal insulation. For example, maximal fall in body temperature at thermoneutrality with the same intrahypothalamic dose of noradrenaline was 1° to 2° C in adult fowls and 3° to 6° C in young chicks.

Qualitatively similar effects were obtained with infusions into the hypothalamus of noradrenaline, an agonist at α-adrenoceptors, as with isoprenaline, an agonist at β-adrenoceptors. Given intraventricularly or into the hypothalamus, equimolar doses of α-methylnoradrenaline had the longest duration of action, noradrenaline the shortest, and isoprenaline was intermediate. The lack of effect of dopamine, except after mebanazine, and the shorter duration of action of noradrenaline compared with isoprenaline and α-methylnoradrenaline, corresponds with the susceptibility of the compounds to monoamine oxidase (Blaschko, 1952). This relation, which involves uptake of the amine into neurones where monoamine oxidase is located, may be coincidental since isoprenaline is not taken up into neurones (Hertting, 1964 ; Ross & Renyi, 1966).

In the mammal there are dopaminergic neurones which terminate in the striatum (Andén, Carlsson, Dahlström, Fuxe, Hillarp & Larsson, 1964). The paleostriatum augmentatum of fowls, homologous to the mammalian striatum (Juorio & Vogt, 1967), also contains a large concentration of dopamine (Spooner & Winters, 1966 ; Juorio & Vogt, 1967). Infusion of dopamine into this area, elicited repetitive movements superimposed on deviation of the head to the contralateral side. Surprisingly, these phenomena were less manifest than with dopamine infusions into the hypothalamus which is immediately posterior to the paleostriatum augmentatum. The long latency for onset of movements after infusions of dopamine is difficult to explain. It is unlikely to be due to the time required for diffusion to sites of action, since latency with infusions into the paleostriatum augmentatum

was just as long as that for the more remote infusions into the hypothalamus. These effects of dopamine were potentiated by pretreating fowls with mebanazine; they were significantly diminished by haloperidol; they were not replicated by noradrenaline infused into the hypothalamus or paleostriatum augmentatum. Repetitive head movements, with or without postural alteration, were noted in rats following intra-striatal injection of dopamine (Fog & Pakkenberg, 1971; Fog, Randrup & Pakkenberg, 1967; Ungerstedt, Butcher, Butcher, Andén & Fuxe, 1969); these effects were potentiated by pretreatment with a monoamine oxidase inhibitor. 'Contraversive' turning was also elicited in rats by unilateral electrical stimulation of the striatum (Arbuthnott & Crow, 1971), presumably due to release of dopamine at striatal nerve endings. Similar phenomena were invoked in cats by application of dopamine to the caudate nucleus (Cools & van Rossum, 1970).

In peripheral tissues, the notion that 3′,5′-cyclic AMP functions as a 'second messenger' for noradrenaline and 'neurohormones' is supported by studies showing that the effects of these substances are mimicked by the addition of exogenous 3′,5′-cyclic AMP (Robison, Butcher & Sutherland, 1968). 3′,5′-cyclic AMP given intraventricularly or into the hypothalamus of fowls was without effect on behaviour, electrocortical activity and body temperature, presumably because it was rapidly destroyed by phosphodiesterase. Following aminophylline, a phosphodiesterase inhibitor, prolonged behavioural and electrocortical sleep developed in 3 of 5 fowls in which 3′,5′-cyclic AMP was infused into the hypothalamus. Some effects of 3′,5′-cyclic AMP are known to resemble those of noradrenaline in the central nervous system. For example, Siggins *et al.* (1971) noted, as with noradrenaline, that iontophoretically applied 3′,5′-cyclic AMP or its dibutyryl derivative, depressed spontaneous discharge of the cerebellar Purkinje cells of rats.

Adenosine, which penetrates membranes more readily than 3′,5′-cyclic AMP, consistently induced sleep in fowls pretreated with aminophylline. Adenosine and 3′,5′-cyclic AMP are capable therefore of emulating, and so may be implicated in, the behavioural and electrocortical effects of catecholamines in fowls. They lacked the hypothermic action of catecholamines, which is presumably mediated via other mechanisms. That the effects of adenosine and 3′,5′-cyclic AMP were manifest only after pretreating fowls with aminophylline, implies that phosphodiesterase is localized postsynaptically as well as presynaptically. There is recent histochemical evidence for this (Breckenridge & Johnston, 1969; Florendo, Greengard & Barrnett, 1970).

The effects of dibutyryl cyclic AMP differed manifestly from those of 3′,5′-cyclic AMP and adenosine. Following its infusion into the hypothalamus or IIIrd ventricle, the fowl became intensely alert, motor activity increased and included 'escape responses', respiratory rate rose to between 90 and 120/min, and body temperature was elevated 2° C or more. With infusions into the hypothalamus, there was in addition deviation of the head to the contralateral side accompanied by large amplitude repetitive excursions of the head and neck from this position. Some of these phenomena had earlier been reported in rats and cats after application of dibutyryl cyclic AMP to similar sites (Gessa, Krishna, Forn, Tagliamonte & Brodie, 1970) except that deviation of the head to the contralateral side developed in cats but not rats and only after its infusion into the amygdala. Generalized convulsions were regularly observed in cats (Gessa *et al.*, 1970; McKean, Peterson & Raghupathy, 1969) and in rats (Gessa *et al.*,

1970), but in fowls the intermittent clonic movements rarely spread to the entire body. Effects in all the species were long-lasting and ended in death or recovery within 24 hours.

Electrocortical changes in fowls were of three kinds: (1) bursts of 6–8 Hz spike activity, (2) regular 1 Hz spike activity and (3) 1–2 Hz, spike and wave activity. Clonic movements occasionally developed in association with the first two patterns but convulsions were infrequent. The bilateral nature of electrocortical changes following infusion of dibutyryl cyclic AMP into the hypothalamus of intact fowls, but their restriction to the ipsilateral hemisphere in *encéphale isolé* preparations, is difficult to explain. Large amplitude 8–10 Hz electrocortical potentials were observed in cats following injection of dibutyryl cyclic AMP into the amygdala (Gessa *et al.*, 1970); these authors comment on the possible significance of 3′,5′-cyclic AMP in epilepsy. The importance of 3′,5′-cyclic AMP in mediating some of the central effects of catecholamines is difficult to assess. The results in fowls with adenosine favour such a role, whereas less crucially, those with the dibutyryl derivative, are against it.

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