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TRYPTAMINES AND SOME OTHER SUBSTANCES AFFECTING WAKING AND SLEEP IN FOWLS

E. MARLEY & G. NISTICÒ[1]

Department of Pharmacology, Institute of Psychiatry, De Crespigny Park, London SE5 8AF

1 Adult fowls (*Gallus domesticus*) with cannulae chronically implanted in the IIIrd cerebral ventricle and various other sites of the brain, received infusions or injections of tryptamines and catecholamines into the brain; effects of and interactions between these substances on behaviour, electrocortical activity and body temperature were studied. Reserpine-induced arousal, was investigated in young and adult fowls.

2 Tryptamine and α-methyltryptamine, given intraventricularly or into the hypothalamus of intact fowls evoked behavioural and bilateral electrocortical arousal, postural changes, elevation of body temperature and tachypnoea; behavioural and bilateral electrocortical arousal were obtained with infusions into the mesencephalon. Ipsilateral electrocortical arousal only, resulted from infusion of α-methyltryptamine into the hypothalamus or mesencephalon of fowl encéphale isolé preparations. The above effects in intact fowls were reduced or replaced by sleep following administration of noradrenaline or α-methylnoradrenaline into the IIIrd ventricle or hypothalamus. Pretreatment of intact fowls with an amine oxidase inhibitor surprisingly attenuated or reversed the excitant effects of intraventricular tryptamine.

3 5-Hydroxytryptamine (hydrogen maleinate, creatinine sulphate or oxalate) given intraventricularly or infused into the hypothalamus, elevated body temperature; tachypnoea and postural changes developed at some stage during the elevation of body temperature. Sleep also was induced, although with the oxalate this was succeeded by marked arousal.

4 Behavioural and electrocortical sleep induced by 5-hydroxytryptamine infused into the hypothalamus were replaced by arousal on infusing tryptamine into the hypothalamus, and *vice versa.*

5 Dexamphetamine infused into the hypothalamus induced drowsiness or sleep which even reversed arousal elicited by systemically administered dexamphetamine.

6 Reserpine-induced arousal was achieved in young and adult fowls pretreated with mebanazine; this arousal was attenuated or replaced by sleep following intraventricular noradrenaline or dopamine but not by 5-hydroxytryptamine nor by noradrenaline or dopamine applied to the hypothalamus. Prenylamine also induced arousal following pretreatment of chicks with mebanazine.

Introduction

Tryptamine, α-methyltryptamine and dexamphetamine given systemically elicit behavioural and electrocortical arousal (Dewhurst & Marley, 1965a, b), and elevate body temperature and oxygen consumption (Allen & Marley, 1967) in fowls. Arousal was assumed to be due to an action on the brain, since intravenous injection of these amines also evoked behavioural and electrocortical arousal in chicken encéphale isolé preparations (Marley & Stephenson, 1971). Brief arousal, followed by longer-lasting sleep, was obtained with intravenous 5-hydroxytryptamine (Hehman, Vonderahe & Peters, 1961; Dewhurst & Marley, 1965b).

Present experiments were undertaken in adult fowls to determine whether intraventricular injection or intracerebral infusion of these amines elicited similar effects to intravenous administration; their site of action was also investigated by infusing the amines into various brain areas. Effects obtained with systemically administered tryptamine, α-methyltryptamine, and 5-hydroxytryptamine, which had been ascribed to central actions, were indeed elicited by their application to the hypothalamus and mesencephalon although

[1] Present address: University Institute of Pharmacology, IInd Faculty of Medicine, Naples, Italy.

this was not the case for amphetamine. A brief account of this work has been published (Marley & Nisticò, 1971).

Behavioural arousal resembling that obtained with amphetamine, has been induced by reserpine in a number of species following pretreatment with an amine oxidase inhibitor (Brodie, Pletscher & Shore, 1956; Chessin, Kramer & Scott, 1957; Graeff, Leme & Silva, 1965; Bueno, Pscheidt & Himwich, 1968). The syndrome, not described hitherto in fowls, was elicited by prenylamine as well as by reserpine. Arousal under these circumstances has been attributed in mammals to release by reserpine within the brain of noradrenaline and/or dopamine (Graeff *et al.*, 1965) or 5-hydroxytryptamine (Chessin *et al.*, 1957). Dopamine, noradrenaline or 5-hydroxytryptamine were therefore given into the brain of fowls, roused by reserpine after mebanazine, to determine whether their administration enhanced or mitigated the syndrome.

Methods

Rhode Island Red fowls of 1.5-2.0 kg and housed at 20°-25°C were used as were 10-16 day old pullets kept in a thermostatically controlled cage at 29°-31°C, ambient temperatures within their thermoneutral ranges.

For operative procedures under halothane anaesthesia and experimental methods, see Marley & Nisticò (1972). Cannulae were implanted stereotactically into the brain under aseptic conditions. Brain coordinates for stereotactic implantation of cannulae not given in Marley & Nisticò (1972), include telencephalon A + 8.0, L + 8.0, V + 9.0; paleostriatum augmentatum A + 9.0, L = 2.5, V + 7.5; mesencephalon A + 4.0, L = 3, V + 3.5 and A + 2.2, L = 1, V + 3.0 (from atlas of van Tienhoven & Juhász, 1962). Fowls were not tested until at least a week after recovery from operative procedures, and thereafter at intervals of at least a week. Cannulae positions were subsequently located by histological examination of the brain. Elevation of body temperature with the tryptamines appeared to have been more readily elicited in fowls with the cannula tip found to be in the anterior rather than in the posterior hypothalamus.

Drugs used were hydrochlorides of *p*-chlorophenylalanine methyl ester, dopamine, (-)-α-methylnoradrenaline, nialamide, (+)-α-methyltryptamine and tryptamine; the oxalate, creatinine sulphate and hydrogen maleinate salts of 5-hydroxytryptamine; dexamphetamine sulphate; mebanazine oxalate, methysergide bimaleate; sodium oxalate, disodium maleinate and creatinine sulphate; prenylamine gluconate (Segontin gluconate 5%, Hoechst); reserpine was dissolved in glacial acetic acid and buffered with 8% NaOH. (−)-Noradrenaline base was dissolved immediately before use in equimolar hydrochloric acid.

Results

Tryptamine

Intraventricular Infusion of tryptamine, 0.5 and 1.0 μmol, evoked intense behavioural and electrocortical alerting within 5 to 10 min in 5 of 7 fowls tested. Rapid jerking movements of the head also developed which periodically escalated into repetitive preening of body feathers. Vocalization, uncommon in adult fowls isolated in the observation box, became frequent, as did pecking. Some 15-20 min after tryptamine the wings were abducted at an angle of about 30° from the trunk, the primary wing feathers diverging and emerging from behind the secondary wing feathers. The tail was extended, and the back became horizontal or even sloped downwards from the tail to the head, replacing the normal slope of the spine. (These changes in the alignment of body and wings are described hereafter as postural changes.) Electrocortical, behavioural and postural changes persisted for 30 to 120 min, depending on dose. Tachypnoea developed almost immediately after tryptamine, respiratory rate increasing from control values of 20 to 30/min up to 100 to 160/min and slowly subsiding over the ensuing 60 to 90 minutes. Increases of body temperature of between 0.5° and 2.0°C, maximum about 60 min after infusion, were noted in 6 of the 7 fowls. When tryptamine, 0.1-0.3 μmol, was infused into the *hypothalamus* of normally alert fowls similar intense arousal and postural changes were produced, except that their onset was preceded by sleep for 5 to 10 minutes. *Mesencephalic* infusion of tryptamine, 0.2 and 0.3 μmol (2 normally alert fowls), also evoked intense electrocortical and behavioural alerting following sedation for 5-10 min, but the postural changes were absent. Infusions into the *paleostriatum augmentatum* of tryptamine, 0.3 μmol (3 fowls) did not affect behaviour, temperature and electrocortical activity.

Antagonism between tryptamine and noradrenaline Effects of tryptamine given into the IIIrd ventricle or hypothalamus were attenuated or antagonized by prior or subsequent infusion of noradrenaline into the hypothalamus. Thus with a fowl in which intraventricular tryptamine 1.0 μmol evoked typical behavioural, electro-

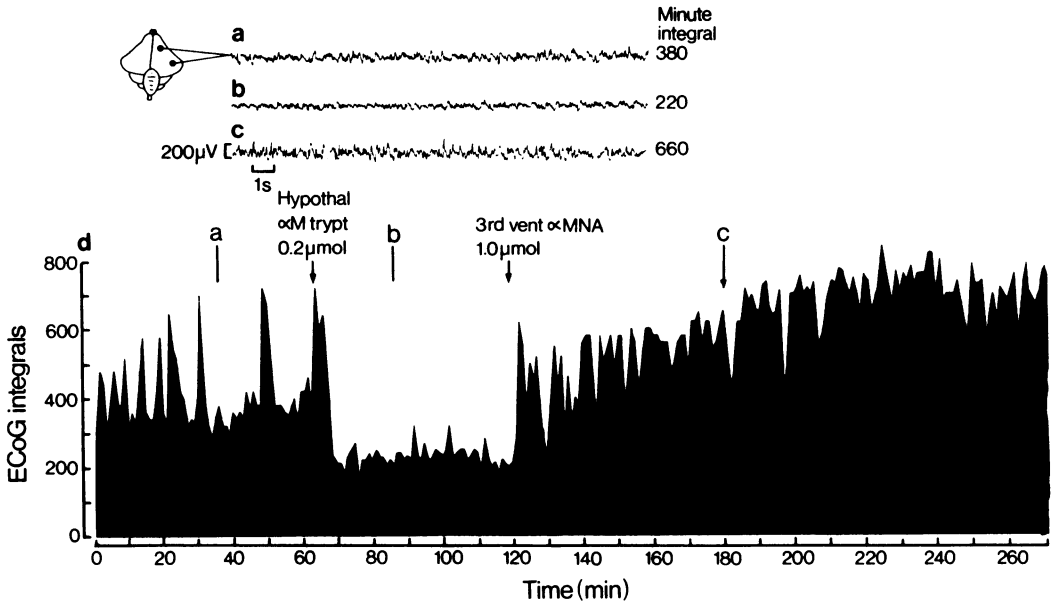

Figure 1 Antagonistic effect in an adult fowl of α-methylnoradrenaline (αMNA) on electrocortical arousal induced by α-methyltryptamine (αMtrypt). (a) Control drowsy electrocortical activity. (b) Electrocortical alert activity following α-methyltryptamine (αMtrypt) (0.2 μmol) infused into the hypothalamus and accompanied by behavioural arousal. (c) Sleep electrocortical activity accompanying behavioural sleep and following α-methylnoradrenaline (1.0 μmol) injected intraventricularly. (d) Integrals corresponding to electrocortical activity showing decrease with electrocortical arousal and increase with electrocortical sleep.

cortical, respiratory and postural effects and increase in body temperature from 40.8°C to 41.5°C, noradrenaline, 0.1 μmol infused into the hypothalamus 45 min later, induced sleep for 2 h, abolished tachypnoea and within the ensuing 30 min, lowered body temperature to 39°C. In another fowl in which noradrenaline (0.1 μmol into the IIIrd ventricle) induced profound behavioural and electrocortical sleep, tryptamine (0.2 μmol) infused into the hypothalamus 60 min later, when the fowl was still deeply asleep, evoked its characteristic effects although these were attenuated. Thus behavioural and electrocortical arousal ensued for 20 min before the fowl reverted again to sleep; respiratory rate increased from 9/min to 60-80/min and there was slight wing abduction.

Monoamine oxidase inhibition The behavioural effects of intraventricular tryptamine were unexpectedly modified, and only the temperature changes potentiated by prior amine oxidase inhibition. Thus, in a normally alert fowl, after nialamide (100 μmol/kg, i.p. 16 h and 1 h previously) tryptamine, 0.5 μmol, instead of producing intense arousal, induced behavioural and electrocortical sleep within 5 min, effects which lasted 3 hours. In two fowls pretreated with mebanazine (100 μmol/kg, i.p. 16 h and 1 h previously in one and 40 h, 16 h and 1 h previously in the other) intraventricular tryptamine, 0.5 μmol, lacked effect on behaviour although previously eliciting intense arousal in the absence of amine oxidase inhibition; body temperature was increased by 1° and 1.5°C for 6 h and 5 h respectively compared with 1 to 2 h before mebanazine.

α-Methyltryptamine in intact fowls

Injection of α-methyltryptamine (0.5 and 1.0 μmol) into the *IIIrd ventricle* (3 fowls) evoked behavioural, electrocortical, respiratory and postural changes within 5-10 min, similar to those after intraventricular tryptamine but lasting up to 4 hours. Infused into the *hypothalamus* (3 fowls) and *mesencephalon* (2 fowls), α-methyltryptamine (0.2 to 0.4 μmol) elicited behavioural and electrocortical arousal (Figure 1) within 3 to 5 min similar to that observed after tryptamine but of longer duration (2 to 3 h); infusions into the *telencephalon* lacked effects.

Antagonism between α-methyltryptamine and noradrenaline or α-methylnoradrenaline The effects of intrahypothalamic α-methyltryptamine

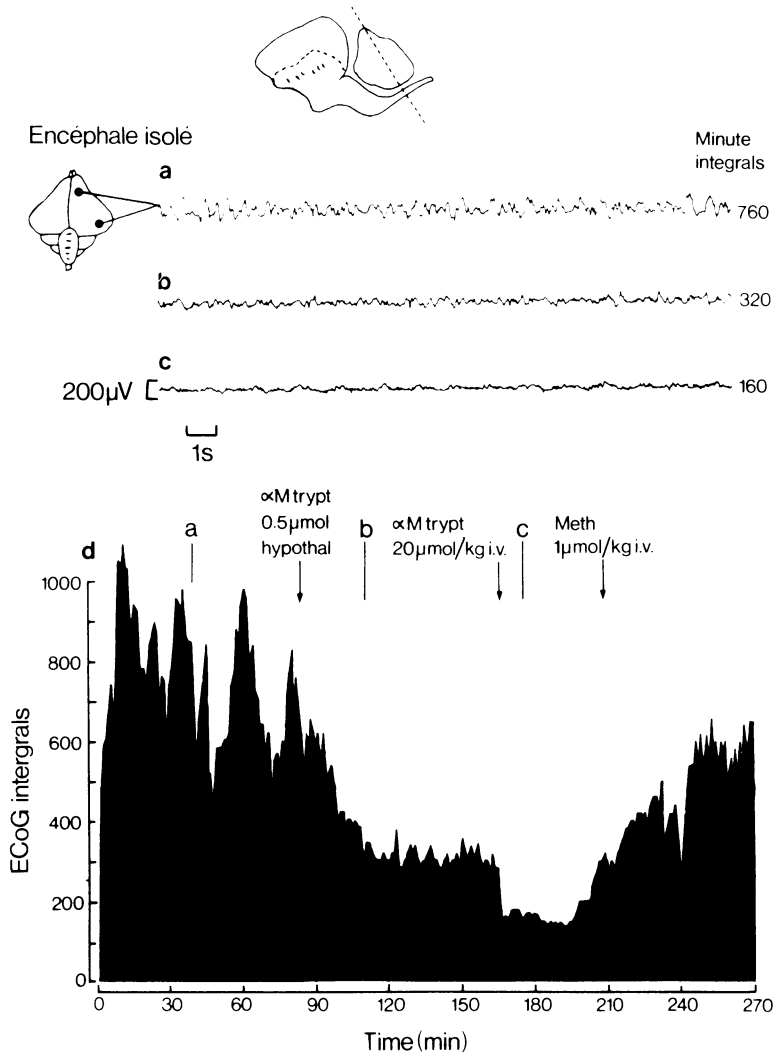

Figure 2 Electrocortical activity in a fowl encéphale isolé preparation (dotted line indicates location of transection) showing electrocortical arousal with α-methyltryptamine (αMtrypt) infused into the hypothalamus (compare b, with control sleep activity in a), with intensification of arousal by intravenous α-methyltryptamine (c). (d) Integrals of electrocortical activity showing reduction of these by α-methyltryptamine and antagonism by methysergide (Meth).

were antagonized by α-methylnoradrenaline (1.0 μmol) given into the IIIrd ventricle, as shown for electrocortical activity in Figure 1. In another fowl, behavioural and electrocortical sleep induced by intraventricular noradrenaline (1.0 μmol), were reversed to behavioural and electrocortical arousal lasting about 2 h following α-methyltryptamine (0.4 μmol) infused into the hypothalamus.

α-Methyltryptamine in fowl encéphale isolé preparations

The effects of α-methyltryptamine were also studied in fowl encéphale isolé preparations which in the absence of drug administration, exhibit sustained behavioural and electrocortical sleep (Marley & Stephenson, 1971). The infusion cannula was implanted into the hypothalamus in 7 fowls, into the mesencephalon in 2, and immediately posterior to the mesencephalon in 2 other fowls.

Unilateral electrocortical desynchronization developing over 20 min, but without behavioural arousal, was elicited by α-methyltryptamine (0.5 μmol) infused into the ipsilateral hypo-

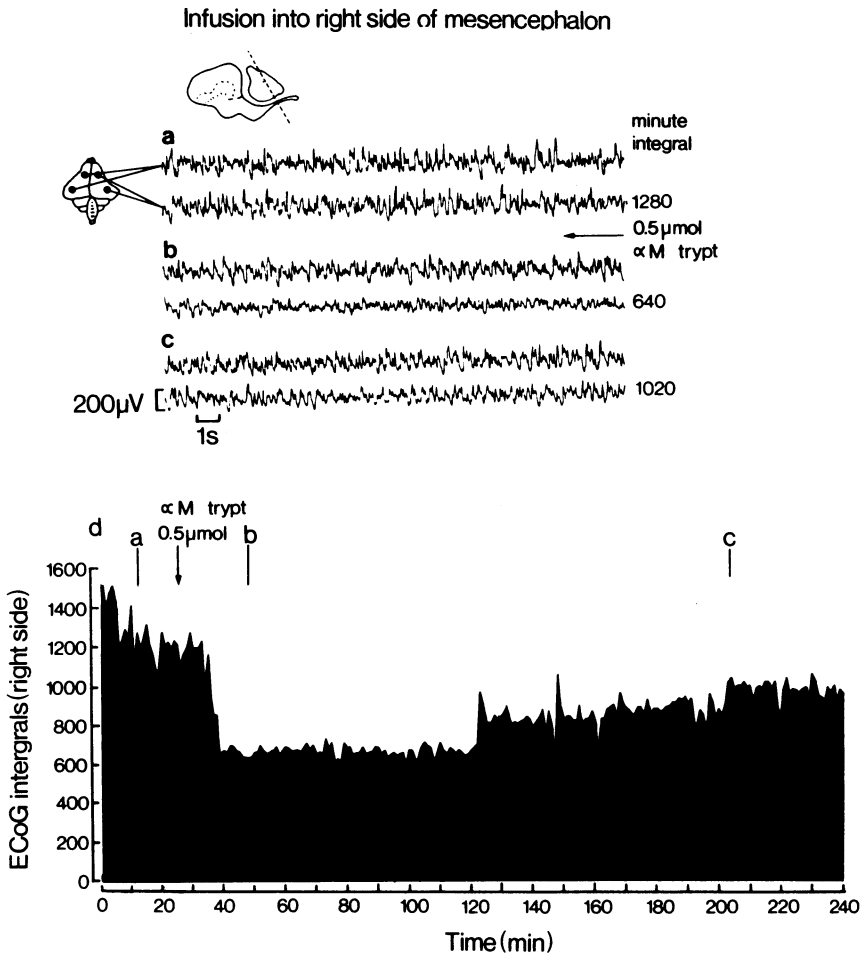

Figure 3 Records of electrocortical activity from a fowl encéphale isolé preparation (dotted line indicates transection) illustrating unilateral electrocortical arousal following α-methyltryptamine (αMtrypt) infused into the mesencephalon. (a) Control sleep electrocortical activity. (b) Electrocortical arousal evident in recording taken from the right cerebral hemisphere 22 min after α-methyltryptamine (0.5 μmol) infused into the ipsilateral mesencephalon, together with halving of electrocortical integrals. (c) Return of sleep electrocortical activity 180 min after α-methyltryptamine with corresponding increase in electrocortical integrals. (d) Integrals corresponding to electrocortical activity.

thalamus (Figure 2b). Subsequent intravenous injection of α-methyltryptamine (20 μmol/kg) intensified electrocortical desynchronization (Figure 2c, d) and extended it to both cerebral hemispheres. These effects, which were accompanied by behavioural arousal, were antagonized by methysergide (1.0 μmol/kg, i.v.), as evident for electrocortical activity from the increase in electrocortical integrals (Figure 2d). Ipsilateral electrocortical desynchronization, developing over 15 min, was much less marked with infusion of α-methyltryptamine (0.5 μmol) into the mesencephalon (Figure 3), and was not elicited with more posterior infusions.

5-hydroxytryptamine

Three forms of 5-hydroxytryptamine and the respective salts were tested, since Roberts & Straughan (1967) reported that the effects of iontophoretically applied 5-hydroxytryptamine varied according to the salt used.

5-hydroxytryptamine oxalate and sodium oxalate, each 0.2 and 0.3 μmol, produced identical effects when infused into the *hypothalamus* (4 fowls). Thus within 5 to 10 min of infusion, the fowl became more behaviourally and electrocortically alert with head shaking, wing abduction, vocaliza-

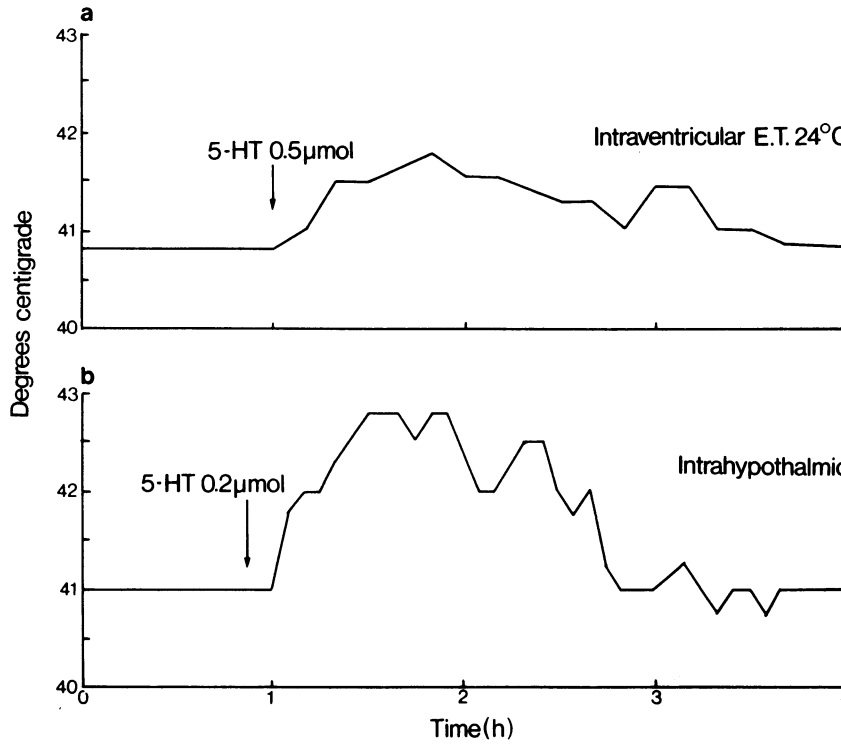

Figure 4 Body temperature responses in same fowl to 5-hydroxytryptamine (5-HT) hydrogen maleinate given (a) intraventricularly, and (b) a week later, into the hypothalamus; environmental temperature (E.T.) was 24° C for both experiments. Note, more intense elevation of body temperature with smaller dose of 5-hydroxytryptamine into the hypothalamus.

tion, 'escape' responses and tachypnoea (60-180/minute). This state lasted 20 to 30 min and was followed by drowsiness for up to 1 hour. Body temperature rose by up to 2.0° C. *Intraventricular* injection of 5-hydroxytryptamine 0.3 to 0.5 μmol, elicited initial drowsiness or sleep for 5 to 30 min (3 normally alert fowls), followed by behavioural and electrocortical arousal for 30 min, together with tachypnoea (60-120/min), vocalization, preening, head-shaking and abduction of the wings from the trunk; body temperature was not significantly altered.

5-hydroxytryptamine hydrogen maleinate. After *hypothalamic* infusion of 5-hydroxytryptamine, 0.1 and 0.2 μmol (3 alert fowls), body temperature increases ranged from 0.75° to 2.8° C, reaching a peak after about 20 min with recovery in 2 to 3 hours (Figure 4b). After about 5 min, 2 of the fowls squatted asleep for 30 min but the third remained alert; once sleep abated, tachypnoea and wing abduction developed. Equimolar doses of disodium maleinate lacked effect when given by this route. In contrast after *intraventricular* disodium maleinate 1.0 μmol, body temperature increased 0.8° and 1.3° C respectively (2 fowls), together with tachypnoea (124/min and 110/min respectively), wing abduction, excitement and escape responses; body temperature and excitation subsided, after 35 minutes. In 4 normally alert fowls, given *intraventricular* 5-hydroxytryptamine (0.5 and 1.0 μmol), body temperature increased over 30 min up to 1° C with recovery in 2 to 3 h (Figure 4a); sleep developed within 5 min and lasted 30 min with tachypnoea and wing abduction appearing once the fowls were alert.

5-hydroxytryptamine creatinine sulphate (0.1 and 0.2 μmol) infused into the *hypothalamus* of 2 fowls evoked sleep, commencing after 5 min and lasting 2 to 3 h, although body temperature was unaltered; sleep was also induced by equimolar infusions of creatinine sulphate (2 fowls) into the hypothalamus. *Intraventricular* 5-hydroxytryptamine, 0.5 μmol, induced sleep but lacked temperature effects. 5-hydroxytryptamine, 1.0 μmol, induced sleep within 5-10 min which lasted

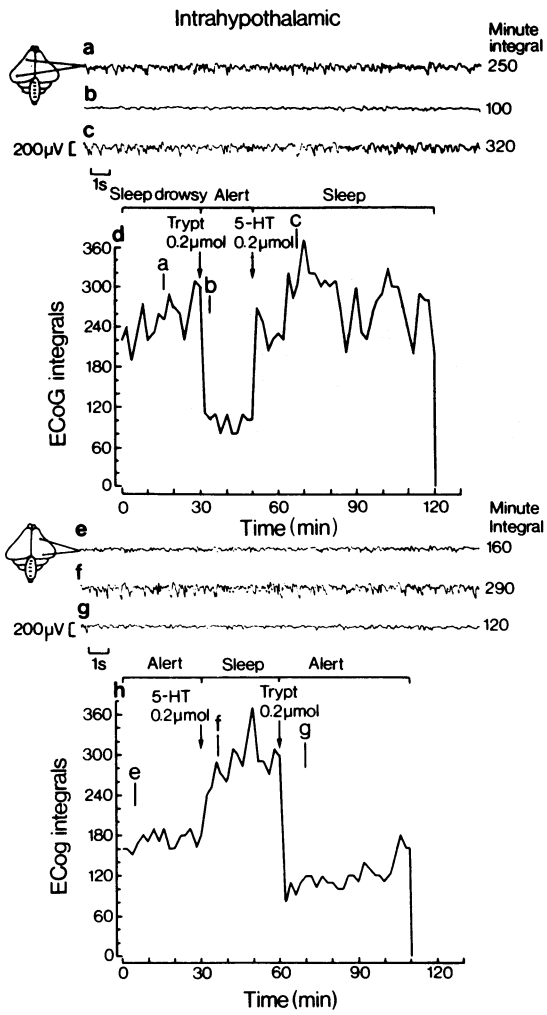

Figure 5 Records of electrocortical activity with corresponding integrals of electrocortical activity in two fowls (a-d and e-h, respectively) to demonstrate that arousal evoked by tryptamine (Trypt) was abolished and replaced with sleep induced by 5-hydroxytryptamine (5-HT), and *vice versa*. (a) Control sleep electrocortical activity. (b) Alert electrocortical activity 4 min after tryptamine, 0.2 μmol, infused into the hypothalamus, accompanying behavioural arousal. (c) Sleep-like electrocortical activity 19 min after 5-hydroxytryptamine, 0.2 μmol, infused into the hypothalamus and associated with behavioural sleep. (d) Electrocortical integrals demonstrating marked reduction during tryptamine-evoked arousal and subsequent rapid and substantial increase following 5-hydroxytryptamine. (e) Control alert electrocortical activity. (f) Sleep-like electrocortical activity 6 min after 5-hydroxytryptamine, 0.2 μmol, infused into the hypothalamus, accompanying behavioural sleep. (g) Alert electrocortical activity 10 min after tryptamine, 0.2 μmol, infused into the hypothalamus and associated with behavioural arousal. (h) Electrocortical integrals exhibiting marked increase during 5-hydroxytryptamine-induced sleep and subsequent rapid and substantial decrease following tryptamine.

55 min; body temperature was elevated 1.0°C with recovery after 160 min; heat-loss mechanisms were activated since wing abduction developed after 105 min, lasting 50 min, accompanied by tachypnoea (respiratory rate 42-86/min) for 20 minutes.

Antagonism between tryptamine and 5-hydroxytryptamine. Since tryptamine and 5-hydroxytryptamine had opposite effects on behaviour and electrocortical activity, they were conceivably acting on different receptors or neuronal systems. Experiments were therefore undertaken to ascertain whether, in the same fowl, arousal due to tryptamine was abolished and replaced by sleep following infusion of 5-hydroxytryptamine hydrogen maleinate, into the hypothalamus, and *vice versa*. The first of the 3 fowls tested, initially slept or drowsed (electrocortical activity and integrals, Figure 5a, d). Tryptamine, 0.2 μmol, infused into the hypothalamus then evoked electrocortical arousal within 4 min (Figure 5b), together with reduction of electrocortical integrals from between 190-310/min to about 80-100/min (Figure 5d); this was accompanied by behavioural arousal, wing abduction, vocalization, tachypnoea and pecking. 5-Hydroxytryptamine, 0.2 μmol, infused into the hypothalamus 20 min subsequently (a time when behavioural and electrocortical arousal would have persisted a further 20-40 min, see Figure 5h), replaced the alert state within 5 min by one of sleep, pecking and vocalization abating. The electrocortical record (Figure 5c) and increase in integrals (Figure 5d) represent changes 18 min after 5-hydroxytryptamine. In contrast, the second fowl was initially electrocortically (Figure 5e, h) and behaviourally alert but 5-hydroxytryptamine, 0.2 μmol, infused into the hypothalamus induced electrocortical sleep within 6 min (Figure 5f), electrocortical integrals increasing from between 150-190/min, to a peak of 370/min (Figure 5h); behavioural sleep also developed. Thirty minutes later, tryptamine, 0.2 μmol, infused into the hypothalamus elicited electrocortical arousal (Figure 5g) and diminished electrocortical integrals to approximately 100/min (Figure 5h); sleep was replaced by behavioural arousal accompanied by pecking, vocalization and wing abduction. Similar results were obtained in a third fowl tested.

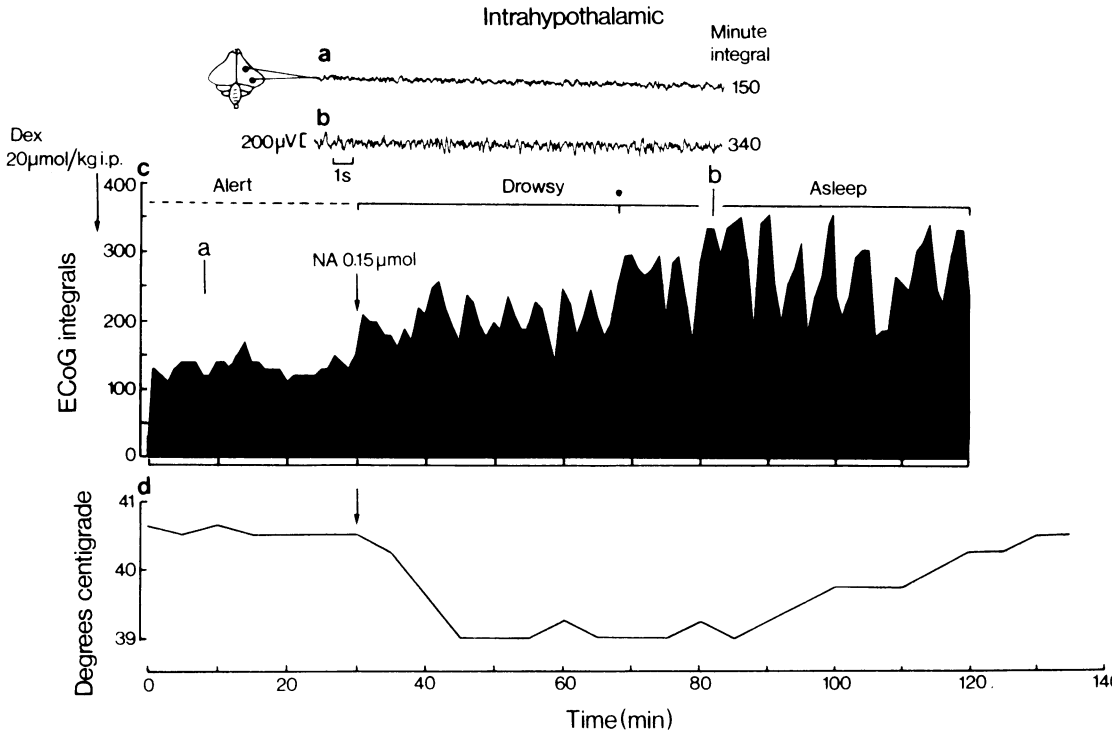

Figure 6 Antagonistic effects in an adult fowl of noradrenaline (NA) on electrocortical arousal induced by dexamphetamine (Dex). (a) Control electrocortical activity after dexamphetamine (20 μmol/kg, i.p.) given 40 min previously. (b) Electrocortical sleep activity 55 min after noradrenaline (0.15 nmol) infused into the hypothalamus. (c) Histogram of integrated electrocortical activity showing progressive increase following intrahypothalamic infusion of noradrenaline, as the fowl becomes drowsy and then asleep. (d) Fall of body temperature up to 1.5° C following noradrenaline.

Dexamphetamine

Dexamphetamine, 0.2 to 0.5 μmol, infused into the *hypothalamus* (3 fowls), evoked behavioural and electrocortical drowsiness or sleep of 1 to 2 h duration. Interestingly, behavioural and electrocortical arousal elicited in a fowl by intraperitoneal injection of dexamphetamine 20 μmol/kg (a dose sufficient to sustain arousal for 60 min) was replaced 10 min later by behavioural and electrocortical sleep for 55 min, almost immediately following dexamphetamine, 0.8 μmol, infused into the hypothalamus.

Antagonism between dexamphetamine and either noradrenaline or α-methylnoradrenaline Two fowls were tested, intraperitoneal dexamphetamine (20 μmol/kg) being given to induce behavioural and electrocortical arousal. Noradrenaline (0.15 μmol) in one fowl and α-methylnoradrenaline (0.1 μmol) in the other, were infused respectively 40 and 30 min later into the hypothalamus. Both fowls became drowsy and then profoundly asleep, sleep lasting at least 3 h and the alert electrocortical activity (Figure 6a) changing to large amplitude, slow frequency potentials (Figure 6b) electrocortical integrals increasing two to threefold (Figure 6c) and body temperature falling 1.4° C (Figure 6d).

Arousal induced by reserpine following mebanazine

Adult fowls After pretreatment with mebanazine (100 μmol/kg, i.m. 18 h and 2 h previously), reserpine (20 μmol/kg, i.m.) was given to thirteen fowls. In ten of these, reserpine induced behavioural and electrocortical drowsiness or sleep lasting 45-120 min followed by more than 6 h of intense arousal (Figure 7a-c). During the drowsy period, the fowls stood immobile with lowered wings and flexed tail whereas during the ensuing alert state, the fowls evinced marked locomotor activity, pecked the walls and floor of the

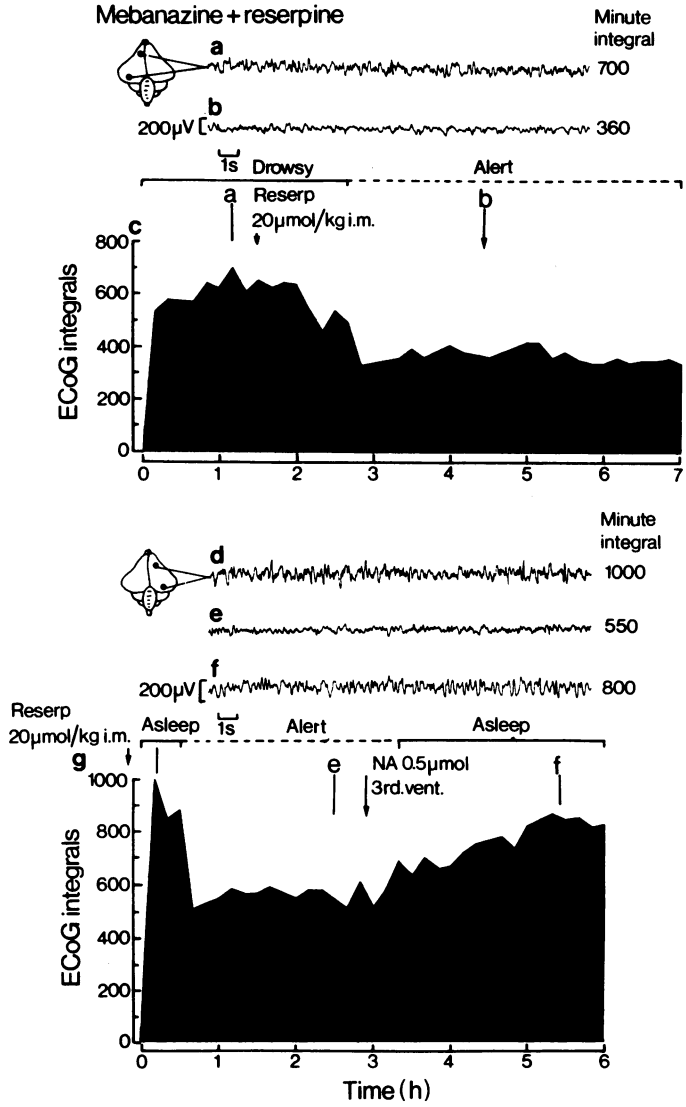

Figure 7 Records of electrocortical activity with corresponding integrals of electrocortical activity in two fowls (a-c and d-g respectively) pretreated with mebanazine (100 μmol/kg, i.m. 18 h and 2 h previously). (a) Control sleep electrocortical activity. (b) Alert electrocortical activity 3 h after reserpine (Reserp) (20 μmol/kg, i.m.). (c) Electrocortical integrals showing marked reduction during reserpine-induced arousal and persisting for 5.5 h when the experiment was terminated. (d) Control sleep electrocortical activity in another fowl pretreated with mebanazine as above and followed by reserpine (20 μmol/kg, i.m.) (e) Alert electrocortical activity during reserpine-induced arousal. (f) Sleep electrocortical activity associated with behavioural sleep 2.5 h following noradrenaline (NA 0.5 μmol) injected into the third ventricle. (g) Electrocortical integrals showing marked reduction during reserpine-induced arousal, followed by increase associated with sleep evoked by intraventricular noradrenaline.

observation box and vocalized, unusual in the test situation; the wings were abducted about 60° from the trunk and respiratory rate rose to 200/minute. This alert phase was absent in the remaining 3 fowls. Body temperature changes (±0.75°C) gave no indication as to which amine was involved in the syndrome, and indeed occurred whether reserpine induced arousal or not.

Noradrenaline administration In 3 fowls with reserpine-induced arousal, intraventricular noradrenaline (0.5 or 1.0 μmol) replaced within 10 to

15 min the intensely alert behavioural and electrocortical activity by electrocortical (Figure 7d-g) and behavioural sleep lasting 4 h or more; the fowls slept squatting or standing. Tachypnoea (up to 200/min) which had developed, disappeared during sleep induced by noradrenaline. In contrast, noradrenaline (0.1 or 0.2 μmol) infused into the hypothalamus of 3 fowls did not diminish arousal induced by reserpine. The long lasting fall of body temperature of 1.5°-2.0°C which follows intraventricular or intrahypothalamic administration of noradrenaline in adult fowls after mebanazine (Marley & Nisticò, 1972) was not observed, body temperature varying ±0.5°C, except in one fowl that died within 24 h of the experiment, in which body temperature rose from 41° to 45.25°C.

Dopamine administration Three fowls were pretreated with mebanazine, 100 μmol/kg, i.m. 18 and 2 h prior to reserpine 20 μmol/kg, i.m. Once behavioural arousal had developed and was intense, dopamine (0.5, 1.0, 2.0 μmol respectively) was given into the IIIrd ventricle. Sedation was apparent in 3 to 10 min, followed by behavioural sleep lasting 2-3 h during which the fowls stood in a 'tripod' position with lowered wings, or squatted; tachypnoea (180-200/min), which accompanied reserpine arousal was slowed to 80-100/minute. Despite behavioural sleep, electrocortical activity was of the alert pattern following the 0.5 and 1.0 μmol doses, but electrocortical sleep lasting about 3 h was induced by the 2.0 μmol dose. Temperature changed ±0.5°C following reserpine and dopamine. Once sleep due to dopamine waned, the fowl reverted to intense alertness. Dopamine (0.1 and 0.2 μmol) infused into the hypothalamus of 2 fowls, failed to antagonize arousal evoked by reserpine after mebanazine.

5-hydroxytryptamine In contrast to noradrenaline and dopamine, *intraventricular* 5-hydroxytryptamine hydrogen maleinate (0.25, 0.5 and 1.0 μmol) in 4 fowls did not diminish behavioural and electrocortical arousal induced by reserpine.

Young chickens Six 1-16 day chicks pretreated with mebanazine (20.0 μmol/100 g, i.p. 16 h and 2 h previously) were given reserpine (2.0 μmol/100 g, i.p.). Following initial sleep, a similar syndrome developed as described for adult fowls above, but was not obtained in 6 chicks administered reserpine only. Prenylamine, like reserpine, depletes the avian brain of noradrenaline, dopamine and 5-hydroxytryptamine (Juorio & Vogt, 1967), so this also was tested. Prenylamine (24 μmol/100 g, i.p.) evoked an arousal syndrome in 8 of 12 chicks pretreated with mebanazine (20 μmol/100 g, i.p. 16 h and 2 h previously); prenylamine alone lacked effect. Bueno *et al.* (1958) suggested that in mammals, arousal after reserpine and an amine oxidase inhibitor was related not to the absolute concentration of brain amines but to an increased 5-hydroxytryptamine : noradrenaline ratio. Experiments were repeated therefore after pretreating chicks with *p*-chlorophenylalanine, a tryptophan hydroxylase inhibitor (Koe & Weissman, 1966). Chicks (10-16 days) were given *p*-chlorophenylalanine (120 μmol/100 g, i.p. daily on 3 successive days) and mebanazine (20 μmol/100 g, i.p.) 16 h and 2 h prior to reserpine (2.0 μmol/100 g, i.p.) and observed for 4 h; a control group, pretreated as above with *p*-chlorophenylalanine, was given reserpine only. The control group squatted asleep, but in contrast those given mebanazine became extremely alert after 30-40 minutes. Arousal lasting 75-135 min, was identical to that obtained with dexamphetamine or α-methyltryptamine viz, pecking, twittering and increased initial motor activity subsiding as postural changes developed; tachypnoea (60-80/min) also developed. Once arousal abated, the chicks stood or squatted, asleep. At the end of the 4 h, chicks pretreated with mebanazine were alive, whereas 5 of the controls were dead.

Discussion

Tryptamine and α-methyltryptamine given intraventricularly or infused unilaterally into the hypothalamus of fowls evoked effects similar to their systemic administration viz, behavioural and bilateral electrocortical arousal, tachypnoea and postural changes, except that effects of these tryptamines were longer-lasting when applied centrally and effective doses were smaller. Behavioural and bilateral electrocortical arousal, but not postural changes and tachypnoea, were also induced by unilateral infusions of tryptamine or α-methyltryptamine into the mesencephalon. That tryptamine receptors, capable of mediating arousal by systemically applied agonists, were located in these areas was evident from brain transection experiments (Marley & Stephenson, 1971). 5-Hydroxytryptaminergic neurones terminate profusely in the chicken hypothalamus and mesencephalon (Ikeda & Gotoh, 1971) and the area is rich in 5-hydroxytryptamine (Juorio & Vogt, 1967); presumably tryptamine and α-methyltryptamine act postsynaptically in these areas. In favour of this idea, their effects when given into the brain were prevented by methyser-

gide (Nisticò, unpublished observations) an antagonist of 5-hydroxytryptamine and tryptamine. Infusion of tryptamines into telencephalic cholinoceptive sites and into dopaminergic areas (paleostriatum augmentatum) were without effects.

Continuity of the brain with the spinal cord was crucial for materialization of all the above phenomena, since ipsilateral electrocortical desynchronization only was induced by infusing α-methyltryptamine unilaterally into the hypothalamus or mesencephalon of the encéphale isolé, although behavioural and bilateral electrocortical arousal were obtained following intravenous α-methyltryptamine in these preparations. An explanation for this is that in the encéphale isolè, inadequate recruitment of neurones for arousal was achieved by unilateral infusions of α-methyltryptamine in the absence of sensory input from the spinal cord, but that arousal after intravenous α-methyltryptamine accrued because of the bilateral and therefore greater recruitment of neurones. Certainly, input from the spinal cord appears more important for spontaneous arousal in fowls than in mammals, since behavioural and electrocortical sleep are uninterrupted in fowl acute encéphale isolé preparations, in the absence of drug administration (Marley & Stephenson, 1971), whereas mammalian acute preparations alternate between waking and sleeping.

5-Hydroxytryptamine given into the IIIrd ventricle or infused into the hypothalamus, induced behavioural and electrocortical sleep, although sleep was less intense than that with equimolar or even smaller doses of noradrenaline. Body temperature was elevated, although not constantly, with activation of heat-loss mechanisms once sleep abated. More significance was attached to results with the hydrogen maleinate than with other 5-hydroxytryptamine salts tested (despite intraventricular disodium maleinate provoking short-lasting behavioural arousal and elevating body temperature) because the maleinate ion, unlike the creatinine sulphate, did not influence response of cat cortical or brain-stem neurones to 5-hydroxytryptamine (Roberts & Straughan, 1967; Bradley, 1968). 5-Hydroxytryptamine had opposite behavioural and electrocortical effects to tryptamine and α-methyltryptamine. This, together with the fact that in the same fowl, tryptamine-induced arousal could be replaced by 5-hydroxytryptamine-induced sleep, and *vice versa,* implied that actions on different types of receptor was feasible. The existence of more than one type of receptor for tryptamines was proposed from studies with smooth muscle preparations (Gaddum & Picarelli, 1957), a view extended to the central nervous system (Vogt, 1968). Evidence supporting the latter came from Clineschmidt & Lotti (1974), who found certain indoleamine antagonists exhibited large differences in potency towards central effects of parenterally administered 5-hydroxytryptophan or tryptamine. However, since larger doses of tryptamine and 5-hydroxytryptamine evoked similar increases of body temperature in fowls, partial agonistic effects of the one substance on the other type of receptor has also to be countenanced.

Catecholamines infused into the hypothalamus of young (Marley & Stephenson, 1970) and adult fowls (Marley & Nisticò, 1972) induce behavioural and electrocortical sleep and lower body temperature. Noradrenaline and α-methylnoradrenaline infused into the hypothalamus, antagonized the central excitant effects of intraventricularly administered tryptamine or α-methyltryptamine, replacing them by behavioural and electrocortical sleep and even reversing the rise in body temperature so that a fall ensued. This antagonism between the two different groups could be regarded as the resultant of their opposite effects. Interactions between 5-hydroxytryptamine and noradrenaline on body temperature were not studied although it is reasonable to assume that though both induced sleep, their effects on body temperature would have been antagonistic. Opposite effects of these amines on body temperature have been noted in a number of species (see Bligh, 1973, for references), although there is no agreed interpretation of their mode of production.

Dexamphetamine given intravenously to young and adult fowls evoked behavioural and electrocortical arousal (Key & Marley, 1962) and elevated body temperature (Allen & Marley, 1967), effects identical to those of tryptamine or α-methyltryptamine and prevented or abolished by a 5-hydroxytryptamine and tryptamine antagonist, methysergide (Dewhurst & Marley, 1965a); effects of dexamphetamine on operant pecking were not prevented by methysergide but by a noradrenaline antagonist, phenoxybenzamine (Marley & Morse, 1967). Thus, dexamphetamine appears to have at least two central modes of action in fowls, a predominant one on tryptamine receptors and another mediated via noradrenaline release. Dexamphetamine given into the brain appeared also to have two different actions. Thus in adult fowls, intraventricular dexamphetamine, 0.5 μmol, elicited slight excitement and 5.0 μmol, even convulsions (Grunden & Marley, 1970), whereas infused into the hypothalamus it induced sleep, to the extent of antagonizing arousal elicited by dexamphetamine given intraperitoneally. The latter results could be ascribed to release of hypothalamic noradrenaline by an intraneuronal

action of dexamphetamine, associated with impaired re-uptake of liberated noradrenaline (see Glowinski & Axelrod, 1966, for references). Compatible with this notion, noradrenaline or α-methylnoradrenaline infused into the hypothalamus, replaced the central excitant effects obtained with dexamphetamine injected intraperitoneally by behavioural and electrocortical sleep. Interestingly, even the excitant effects of tryptamine infused into the hypothalamus were preceded by brief drowsiness or sleep. After pretreating fowls with mebanazine, these excitant effects of intrahypothalamic tryptamine were attenuated or replaced by sleep (parenteral tryptamine under these circumstances evoked sustained arousal; Marley, 1968). Release by tryptamine of hypothalamic 5-hydroxytryptamine, which could cause sleep, may well account for these findings. Fuxe & Ungerstedt (1968) found in rats pretreated with a monoamine oxidase inhibitor, that tryptamine enhanced the rate of disappearance of 5-hydroxytryptamine from 5-hydroxytryptaminergic neurones and inferred that tryptamine could act presynaptically by releasing extragranular 5-hydroxytryptamine.

Behavioural, electrocortical, postural and respiratory changes resembling those obtained with dexamphetamine, tryptamine or α-methyltryptamine, given intravenously, or tryptamine and α-methyltryptamine infused into the hypothalamus, were induced in chickens given reserpine or prenylamine following pretreatment with mebanazine. The syndrome induced by reserpine was antagonized by intraventricular noradrenaline or dopamine but not by either of these substances infused into the hypothalamus, or by intraventricular 5-hydroxytryptamine. Should central noradrenaline or dopamine release by reserpine be involved in this syndrome, then our results indicate that it was unlikely to be from stores of these amines in brain areas accessible to catecholamines from the IIIrd ventricle. If, as suggested by Bueno *et al.* (1968) arousal was due to an increased ratio of brain 5-hydroxytryptamine to noradrenaline, then intraventricular catecholamines would redress this balance and even sleep ensue, as occurred in the fowls. In conflict with this thesis, the syndrome was still obtained with reserpine after doses of *p*-chlorophenylalanine which reduce brain 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in chickens to about 30% of normal (Schrold & Squires, 1971) and yet do not ostensibly interfere with catecholamine synthesis (Modigh & Svensson, 1972).

Finally, it is tempting to speculate on the relation of ion concentration and therefore ion movement at synapses, to the central effects of drugs. Behavioural arousal and elevation of body temperature similar to those evoked by tryptamine, α-methyltryptamine and dexamphetamine were elicited with intraventricular disodium maleinate. Similarly, as noted by Artunkal & Marley (unpublished observations) for fowls, the hydrochlorides of sodium or potassium, given into the brain in concentrations exceeding those normally present, induced arousal with elevation of body temperature; in contrast, centrally applied calcium (as the chloride) elicited effects simulating those of catecholamines, viz, sleep and lowering of body temperature.

References

ALLEN, D.J. & MARLEY, E. (1967). Effect of sympathomimetic and allied amines on temperature and oxygen consumption. *Br. J. Pharmac. Chemother.*, **31**, 290-312.

BLIGH, J. (1973). *Temperature regulation in mammals and other vertebrates*, pp. 133-152. Amsterdam: North-Holland Publishing Co.

BRADLEY, P.B. (1968). Synaptic transmission in the Central Nervous System and its Relevance to Drug Action. *Int. Rev. Neurobiol.* ed. Pfeiffer, C.C. & Smythies, J.R., pp. 1-56. New York: Academic Press.

BRODIE, B.B., PLETSCHER, A. & SHORE, P. (1956). Possible role of serotonin in brain function and in reserpine action. *J. Pharmac. exp. Ther.*, **116**, 9.

BUENO, J.R., PSCHEIDT, G.R. & HIMWICH, H.E. (1968). Interactions of monoamine oxidase inhibitors and reserpine: an EEG, gross behavioral and biochemical analysis. *Psychopharmacologia (Berl).*, **12**, 400-413.

CHESSIN, M., KRAMER, E.R. & SCOTT, C.C. (1957). Modifications of the pharmacology of reserpine and serotonin by iproniazid. *J. Pharmacol. exp. Therap.*, **119**, 453-460.

CLINESCHMIDT, B.V. & LOTTI, V.J. (1974). Indoleamine antagonists: relative potencies as inhibitors of tryptamine–and 5-hydroxytryptophan–evoked responses. *Br. J. Pharmac.*, **50**, 311-313.

DEWHURST, W.G. & MARLEY, E. (1965a). The effects of α-methyl derivatives of noradrenaline, phenethylamine and tryptamine on the central nervous system of the chicken. *Br. J. Pharmac. Chemother.*, **25**, 682-704.

DEWHURST, W.G. & MARLEY, E. (1965b). Action of sympathomimetic and allied amines on the central nervous system of the chicken. *Br. J. Pharmac. Chemother.*, **25**, 705-727.

FUXE, K. & UNGERSTEDT, U. (1968). Histochemical studies on the effects of (+)-amphetamine, drugs of the imipramine group and tryptamine on central catecholamine and 5-hydroxytryptamine neurones

after intraventricular injection of catecholamines and 5-hydroxytryptamine. *Eur. J. Pharmac.*, **4**, 135-144.

GADDUM, J.H. & PICARELLI, Z.P. (1957). Two kinds of tryptamine receptor. *Br. J. Pharmac. Chemother.*, **12**, 325-328.

GLOWINSKI, J. & AXELROD, J. (1966). Effects of drugs on the disposition of H^3-norepinephrine in the rat brain. *Pharm. Rev.*, **18**, 775-786.

GRAEFF, F.G., LEME, J.G. & SILVA, M.R.e. (1965). Role played by catechol and indolamines in the central actions of reserpine after monoamine oxidase inhibition. *Int. J. Neuropharmac.*, **4**, 17-26.

GRUNDEN, L.R. & MARLEY, E. (1970). Effects of sympathomimetic amines injected into the third cerebral ventricle in adult chickens. *Neuropharmacology*, **9**, 119-128.

HEHMAN, B.S., VONDERAHE, A.R. & PETERS, J.J. (1961). Effect of serotonin on behaviour, electrical activity of the brain, and seizure threshold of the newly hatched chick. *Neurology*, **11**, 1011-1016.

IKEDA, H. & GOTOH, J. (1971). Distribution of monoamine-containing cells in the central nervous system of the chicken. *Jap. J. Pharmac.*, **21**, 763-784.

JUORIO, A.V. & VOGT, M. (1967). Monoamines and their metabolites in the avian brain. *J. Physiol. Lond.*, **189**, 489-518.

KEY, B.J. & MARLEY, E. (1962). The effect of sympathomimetic amines on behaviour and electrocortical activity of the chicken. *Electroenceph. clin. Neurophysiol.*, **14**, 90-115.

KOE, B.K. & WEISSMAN, A. (1966). *p*-Chlorophenylalanine: a specific depletor of brain serotonin. *J. Pharmac. exp. Ther.*, **154**, 499-516.

MARLEY, E. (1968). Pharmacological studies. In: *Studies in Psychiatry*, ed. Shepherd, M. & Davies, D.L., pp. 253-288. Oxford: University Press.

MARLEY, E. & MORSE, W.H. (1967). Effects of α-methyl derivatives of noradrenaline, phenethylamine and tryptamine on operant conditioning in chickens. *Br. J. Pharmac.*, **31**, 367-389.

MARLEY, E. & NISTICÒ, G. (1971). Effects of tryptamine and catecholamines administered into the brain or third ventricle of adult fowls. *J. Pharmacol. (Paris)*, **2**, 233-234P.

MARLEY, E. & NISTICÒ, G. (1972). Effects of catecholamines and adenosine derivatives given into the brain of fowls. *Br. J. Pharmac.*, **46**, 619-636.

MARLEY, E. & STEPHENSON, J.D. (1970). Effects of catecholamines infused into the brain of young chickens. *Br. J. Pharmac.*, **40**, 639-658.

MARLEY, E. & STEPHENSON, J.D. (1971). Actions of dexamphetamine and amphetamine-like amines in chickens with brain transections. *Br. J. Pharmac.*, **42**, 522-542.

MODIGH, K. & SVENSSON, T.H. (1972). On the role of central nervous system catecholamines and 5-hydroxytryptamine in the nialamide-induced behavioural syndrome. *Br. J. Pharmac.*, **46**, 32-45.

ROBERTS, M.H.T. & STRAUGHAN, D.W. (1967). Excitation and depression of cortical neurones by 5-hydroxytryptamine. *J. Physiol. Lond.*, **193**, 269-294.

SCHROLD, J. & SQUIRES, R.F. (1971). Behavioural effects of d-amphetamine in young chicks treated with *p*-Cl-phenylalanine. *Psychopharmacologia*, **20**, 85-90.

TIENHOVEN, VAN A. & JUHÄSZ, L.P. (1962). The chicken telencephalon, diencephalon and mesencephalon in stereotaxic co-ordinates. *J. comp. Neurol.*, **118**, 185-197.

VOGT, M. (1968). Cerebral tryptamine receptors: functional considerations. *Adv. Pharmac.*, **6B**, 19-25.

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