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THE EFFECTS OF α-METHYL DERIVATIVES OF NORADRENALINE, PHENYLETHYLAMINE AND TRYPTAMINE ON THE CENTRAL NERVOUS SYSTEM OF THE CHICKEN

BY

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The mammalian central nervous system contains noradrenaline (Vogt, 1954) and 5-hydroxytryptamine (Amin, Crawford & Gaddum, 1954), but study of the possible central functions of these amines poses particular problems. One of these is that parenterally injected amines may elicit a central effect by a peripheral action. For example, behavioural and electrocortical arousal is produced not only by amphetamine, which readily penetrates to the brain, but also by catechol amines, which do not readily cross the blood-brain barrier. Although the central nervous system is clearly involved in both instances, the similarity in effect does not indicate whether the primary action of the amine is central, peripheral or both. Indeed, if the blood-brain barrier is circumvented and an apparently potent excitant, such as adrenaline, is given intracisternally (Leimdorfer, 1950) or intraventricularly (Feldberg & Sherwood, 1954) sleep or sedation ensue.

With the mode of action of drugs on cerebral function uncertain, it is not surprising that investigation of central receptor mechanisms has yielded conflicting results. Thus the electrocortical alerting evoked by catechol amines has been attributed on the one hand to an action on central nervous catechol amine α-receptors (Goldstein & Muñoz, 1961) and, on the other, to an action mediated through cardiovascular α-receptors (Capon, 1960). The behavioural effects of catechol amines are thought to be determined predominantly through central nervous α-receptors (Wurtman, Frank, Morse & Dews, 1959; Dews, 1962). Muñoz & Goldstein (1961) considered that the excitant effects of amphetamine were also mediated through central nervous α-receptors. Vane (1960), however, showed that amphetamine acted on tryptamine receptors in the smooth muscle of the rat stomach and suggested that a similar action might take place in the brain. Additional emphasis in this direction was provided by the observation that monoamine oxidase inhibitors, which potentiate central excitants, lead to different effects on the excretion of the enzyme substrates in the urine, since the concentration of tryptamine showed much the greatest increase compared with that of 5-hydroxytryptamine and the metanephrines (Dewhurst, 1961). Any examination therefore of the actions of sympathomimetic amines on the central nervous system should include tests of the effects of tryptamines.

In the present paper the response of the chicken to one central depressant and to two central excitant amines are described. For this purpose, the α-methyl derivatives of noradrenaline, phenylethylamine and tryptamine were used. Their effects were qualitatively similar to those produced by the parent molecules, but changes were much longer lasting and therefore the more easily observed in detail. Pharmacological antagonism was also studied. In subsequent papers, the effect of a larger series of amines and of their precursors in the chicken and in other species will be presented and related to the chemical structure and lipid solubility of the compounds.

Preliminary accounts of this work have been presented (Dewhurst & Marley, 1964, and communication to the British Pharmacological Society, January 1964).

METHODS

Animals. Rhode Island Red pullets aged 1 to 28 days were used. To reduce sample variations, all pullets studied were hatched and reared by the same dealer; the pullets were sired by the same cockerel and all the dams were siblings.

Anaesthesia. For implanting cannulae and electrodes, the chicken was anaesthetized with halothane (Fluothane, I.C.I., in oxygen 1.5% v/v) delivered from a Goldman vaporizer in a semi-closed non-rebreathing system (Marley & Payne 1964). The anaesthetic was given by mouth-piece, rather than by intubation as originally described, for it was found that laryngeal effects of intubation sometimes affected cheeping.

Operative procedures. Cortical recording electrodes were implanted as described by Key & Marley (1961), except that the electrodes were made from 75 cm lengths of enamelled copper wire (S.W.G.36) instead of nicklechrome. The ends of the electrodes were stripped of insulation and placed on the cortical surfaces. To record electromyograms, similar electrodes were placed in the dorsal muscles of the neck and, in some experiments, in the pectoral muscles and in the flexor and extensor muscles of the thigh. The electrode wires were then taken subcutaneously to emerge at the scalp incision

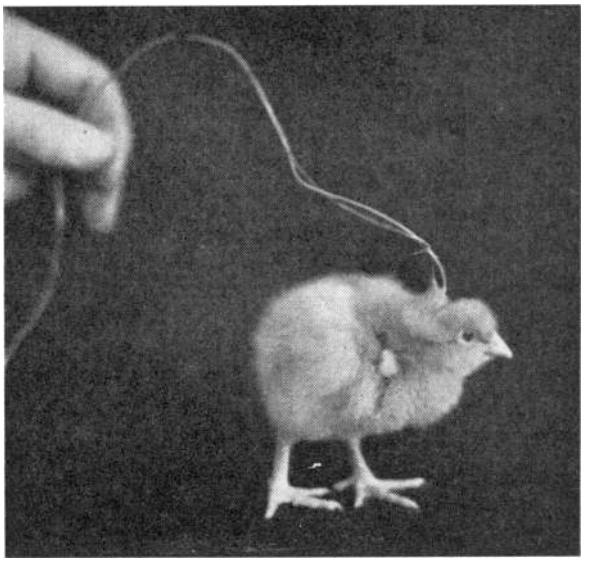

Fig. 1. 4 day-old chicken with polyethylene cannula in right jugular vein, and implanted cortical and muscle recording electrodes.

where they were fixed to the cranium together with the cortical electrodes by Simplex autopolymerizing resin (Dental Fillings Ltd.). A polyethylene tube of 0.5 mm internal diameter and about 75 cm long was filled with heparin-saline (10 mg/ml.) and one end was tied into a jugular vein. The rest of the tubing was brought under the skin to the rear of the scalp incision and fixed to the cranium with resin. The tube and electrodes were twisted together (Fig. 1). The tube had an internal volume of less than 0.2 ml. yet was sufficiently long to allow the chicken to move and feed freely. An adaptor was tied into the end of the tubing so that injections could be given into it.

Postoperative care. Although postanaesthetic recovery was apparently immediate, the chicken was returned to a heated and draught-free ventilated recovery box and kept for at least 24 hr before tests were made. Environmental temperature is critically important for young chickens, and particularly so after operations. The box temperature was therefore maintained at $32 \pm 3°$ C.

Testing arrangements. The chicken was tested in the sound insulated experimental box with one-way glass observation window as previously described (Dewhurst & Marley, 1965a). The leash of polyethylene tubing and electrodes was led to the exterior through a small opening in the box lid. The free ends of the electrodes (distal to the chicken) were bared of insulation and plugged into a junction box connected with the electroencephalograph. Testing began only after the chicken had been in the experimental box for approximately 2 hr. Bipolar recording of electrocortical or electromyographic activity was made with an eight-channel Ediswan, or a Kaiser eight-channel portable, electroencephalograph. Recording and measurement by integration of cheeping, movement, electrocortical and electromyographic activity in the chicken were as described by Dewhurst & Marley (1963, 1965a); integrals were usually recorded for successive 1 min periods. For intravenous injections the drug was contained in 0.1 ml. of 0.9% saline and washed in by 0.2 ml. of saline, at 37 to 40° C. Saline injections were made before and during experiments, since volume changes with intravenous injection could elicit transient drowsiness or behavioural and electrocortical alerting with head-shaking.

Definitions of terms

Postural changes produced by drugs were measured in terms of *the trunk rotation angle* (θ), the angle which the long axis of the trunk made with an imaginary horizontal plane through the tail, and *the tarsal angle* (α), the angle which the tarsus made with the horizontal. Overall effects on posture and movement were also graded into one of four classes. *Grade 0* applied to normal posture. *Grade 1* referred to chickens able to walk but unable to extend fully the lower limbs, so that the animal waddled with bent limbs and horizontal back; wing droop was also present. In *Grade 2* impairment of lower limb extension was more severe and the animal was unable to elevate the trunk from the ground, although still able to move on its belly. In *Grade 3* the chest remained on the ground but the tail was elevated above the rest of the trunk. The beak was held agape and marked contraction of the posterior cervical muscles was accompanied by head retraction.

Vocalization in the young chicken was classified into: *"distress calls"*, which are loud cheeps, repeated at about 1 per sec or less, and commonly elicited by isolation, by cold or by hunger (Collias & Joos, 1953); *twittering*, which is a succession of high-pitched low-intensity calls repeated at 4 to 5 per sec and originally described by Selle (1940) in young chickens given amphetamine; and other types of calling (referred to as *cheeping*).

The drugs studied in this paper had either central depressant or excitant actions.

A *central depressant* was defined as a substance that produced apparent physiological sleep, characterized by a normal sleeping posture with eyes closed, diminished vocalization, movement and electromyographic activity, and 1 to 4 cycles/sec large-amplitude electrocortical potentials.

A *central excitant* was defined as a substance that evoked a pattern of responses comprising increased amounts of vocalization, movement and electromyographic activity, coupled with 10 to 30 cycles/sec small-amplitude electrocortical potentials and, after larger doses, characteristic postural changes.

The *threshold dose* was the smallest dose which gave reproducible depressant or excitant effects on cheeping and electrocortical activity. The threshold dose for the effects on posture applied

only to the excitant amines. The threshold dose was used for comparison of drug potency because it remained reasonably constant irrespective of whether the chicken was initially active or drowsy.

Drugs. These included the hydrochlorides of (+)-α-methylnoradrenaline, (±)-α-methylnoradrenaline, cocaine, (−)-dichloroisoprenaline, (+)- and (−)-α-methyltryptamine, phenoxybenzamine and (±)-pronethalol. Also used were hyoscine hydrobromide, the sulphates of dex- and laevo-amphetamine, chlorpheniramine dimaleate, Hydergine (the methanesulphonate of the dihydro derivative of ergotoxine), methysergide and reserpine. Doses are expressed as μmoles per 100 g of body weight, and injections were intravenous unless otherwise stated.

RESULTS

Central depressant effects of (±)-α-methylnoradrenaline

The average threshold intravenous dose of (±)-α-methylnoradrenaline was 0.025 μmole/100 g (forty-seven chickens) but the effects on cheeping and electrocortical activity were brief. Larger doses (0.1, 0.25, 0.5 and 1.0 μmole/100 g) were therefore used. Fig. 2 shows the effects of the drug (1.0 μmole/100 g) on electrocortical and electromyographic activity in an alert chicken. The fast-frequency 25 μV electrocortical

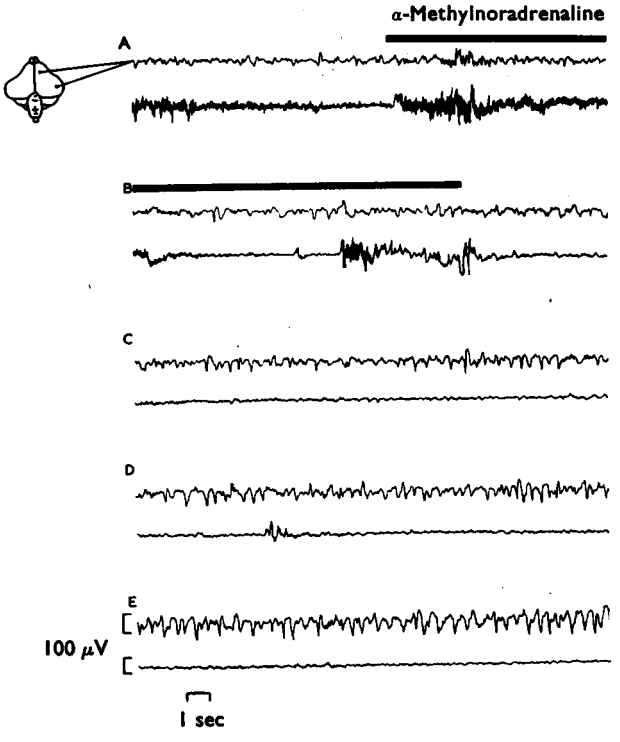

Fig. 2. Effect of (±)-α-methylnoradrenaline on electrocortical and electromyographic activity. Recording from unanaesthetized, unrestrained 7-day-old chicken ; 60 g. From above downwards consecutive records of electrocortical activity (upper trace) and electromyographic potentials (lower trace) over 100 sec. Slow intravenous injection of (±)-α-methylnoradrenaline, 1 μmole/100 g, at bar (A, B). Alert 25 μV electrocortical potentials and 40 μV electromyographic activity (A) change to 150 μV, 2 to 4 cycles/sec, electrocortical potentials and 10 μV electromyographic activity within 80 sec. E: chicken previously alert, now asleep.

potentials and 40 μV activity in the dorsal neck muscles accompanying the alert state (Fig. 2, A), changed during the ensuing 80 sec to 2 to 4 cycles/sec 150 μV electrocortical potentials and 10 μV electromyographic activity (Fig. 2, E). The chicken remained asleep for 30 min with closed eyelids and lowered wings applied closely to the trunk, similar to the position shown in Fig. 4 (C and D). The reduction or abolition in electromyographic activity was observed, not only in the neck muscles, but also in the pectoral and thigh muscles.

Cheeping was diminished or abolished by α-methylnoradrenaline. The effects of three intravenous doses on cheeping and electrocortical activity expressed as integrals are shown in Fig. 3. With the onset of sleep the integrals for cheeping declined and, due

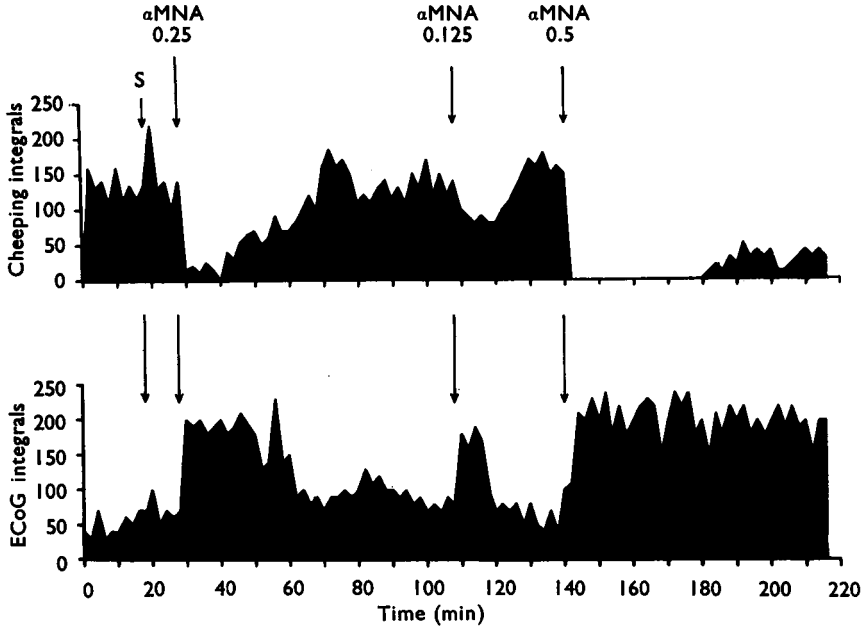

Fig. 3. Histograms of integrals of cheeping and electrocortical activity (ECoG) in a 10-day-old chicken, 70 g. Depressant actions of three doses (in μmoles/100 g) of (±)-α-methylnoradrenaline (αMNA) injected intravenously are shown by decline in cheeping and by increase in amplitude of electrocortical activity with corresponding decrease in cheeping integrals and increase in electrocortical activity integrals. S=0.2 ml. of saline, intravenously.

to the increased amplitude of electrocortical potentials, those for electrocortical activity rose. The duration and intensity of effect depended on the dose, lasting 35, 15 and more than 70 min with the 0.25 μmole/100 g, 0.125 μmole/100 g and 0.5 μmole/100 g doses respectively. Although onset of action was rapid, recovery was gradual and was far from complete at 70 min with the 0.5 μmole/100 g dose. With the larger doses of α-methylnoradrenaline, the time-courses of recovery differed for the different components of the responses. Thus the chicken awoke but cheeping either did not return for a much longer period or, as with the 0.5 μmole/100 g dose in Fig. 3, cheeping returned but the eyelids remained closed, the chicken stayed immobile, and the electrocortical and electromyographic activity was that found during sleep.

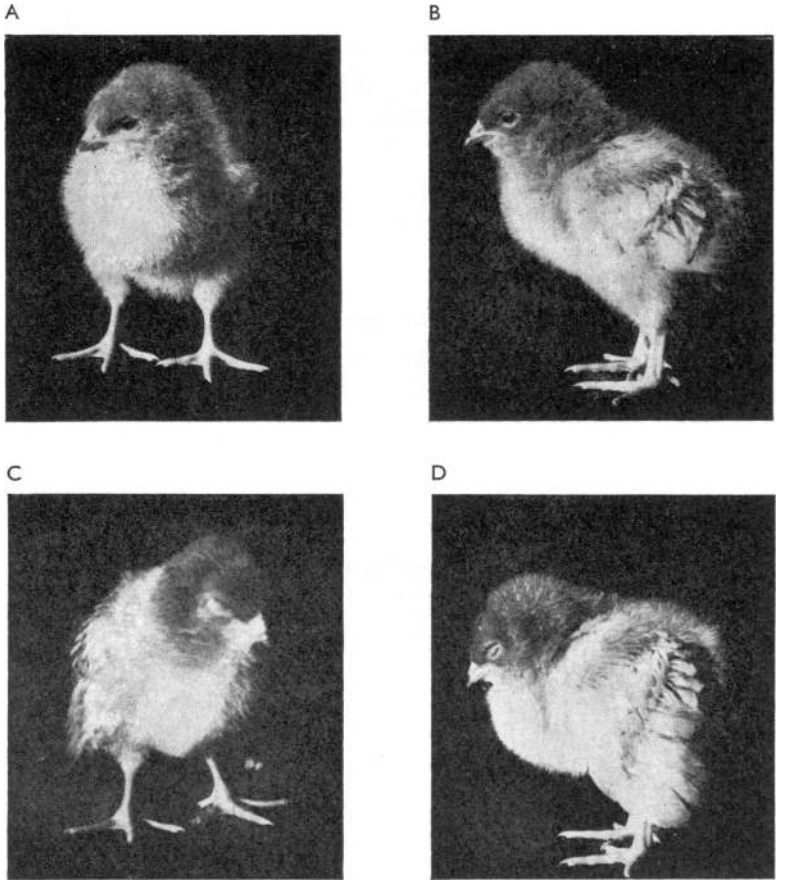

Fig. 4. Effects on posture of (±)-α-methylnoradrenaline (1.0 μmole/100 g, intraperitoneally) in a 9-day-old chicken. Photographs A and B of control alert chicken. C and D after (±)-α-methylnoradrenaline, to show sleeping posture, with head lowered, eyes closed and the lowered wings applied closely to the trunk.

The effects of (±)-α-methylnoradrenaline (1.0 μmole/100 g, intraperitoneally) on posture are shown in Fig. 4. Although examination of the bird revealed diminished muscle tone, postural reflexes were not markedly affected during the sleep produced by α-methylnoradrenaline, since the chicken slept standing. In Fig. 4, D the head is only slightly lowered but usually it was bowed or tucked under the wing; occasionally the bird squatted. However, even though standing, the distance between the two feet was wider than in the alert state, suggesting some impairment of mechanisms maintaining the erect position. With a large intravenous dose (2.0 μmoles/100 g), although the chicken slept standing the head was often bowed so low that the beak touched the floor, the neck and legs forming a tripod.

The chicken could be roused from such sleep by sensory stimuli. The eyelids would open, the nictitating membranes retract and the alert electrocortical pattern return but the arousal was brief and the bird, when left, would be asleep again 30 sec later. If

the chicken moved when roused, then the gait was ataxic when it had received the larger intravenous doses (1.0 or 2.0 μmoles/100 g).

Doses of α-methylnoradrenaline which generally did not produce sleep (0.025 to 0.075 μmole/100 g, intravenously) still diminished activities such as cheeping, pecking and movement.

The central depressant effects of the drug were most conspicuous in 1- to 7-day-old chickens; they declined as the bird matured, and disappeared about the third to fifth week of life.

Central excitant effects of (+)-α-methyltryptamine and of dexamphetamine

These amines had identical effects, although (+)-α-methyltryptamine was about four times more potent in terms of threshold dose than dexamphetamine. The average threshold dose by intravenous injection was 0.12 μmole/100 g for α-methyltryptamine (six chickens) and 0.5 μmole/100 g for dexamphetamine (200 chickens). The rapid onset of the effects of dexamphetamine (1.0 μmole/100 g) on electrocortical and electromyo-

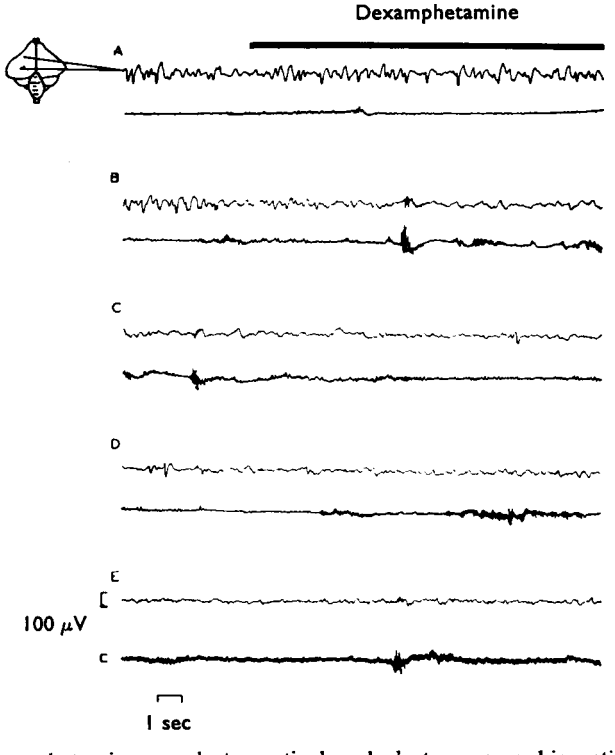

Fig. 5. Effect of dexamphetamine on electrocortical and electromyographic activity. Records from unanaesthetized, unrestrained 5-day-old chicken; 50 g. From above downwards consecutive records of electrocortical activity (upper trace) and electromyographic potentials (lower trace) over 100 sec. Slow intravenous injection of dexamphetamine, 1 μmole/100 g, at bar. Drowsy 150 μV, 4 cycles/sec electrocortical potentials and 20 μV electromyographic activity (A) change to 50 μV fast-frequency electrocortical potentials and 60 μV electromyographic activity within 80 sec (E). Chicken previously drowsy, now alert.

graphic activity in a 5-day-old chicken are shown in Fig. 5. Within 20 sec of the injection, the 2 to 5 cycles/sec 150 μV drowsy electrocortical activity changed to 15 to 30 cycles/sec 25 to 40 μV potentials. The amplitude of the electromyographic activity in the dorsal neck muscles was simultaneously increased, with the appearance of periodic 300 μV potentials due to head shaking (Fig. 5, B), and increased still further over the ensuing 60 sec (Fig. 5, E). The previously drowsy bird was now alert and remained so for over 2 hr. The increased electromyographic potentials were related neither to increased movement nor to cheeping for they preceded both. Moreover, the increased electromyographic potentials were recorded when the bird was immobile. The alerting effect of α-methyltryptamine on electrocortical integrals is shown in Fig. 10; after the intravenous injection of 0.5 μmole/100 g, the integrals of slow electrocortical frequencies declined from an average of 50 to 10 per min and for those for fast electrocortical frequencies, from an average of 20 to 2 per min.

The effects of the excitant amines on posture and on cheeping were of slower onset than those on electrocortical and electromyographic activity and merit more detailed examination.

Postural changes. The normal standing posture of the alert bird is shown in Fig. 6, A. The neck is partially extended so that the bird looks along a horizontal plane level with its head. The long axis of the trunk, that is the line of the dorsolumbar vertebrae, makes an angle of approximately 45° with a horizontal plane through the tail. The wing is opposed to the trunk; the hind-limb shows the customary stance with the long tarsus bone and heel elevated from the ground.

Larger doses were required to produce effects on posture than on electrocortical activity or cheeping. Thus the threshold intravenous dose for eliciting postural changes was 0.5 μmole/100 g for (+)-α-methyltryptamine and 1.0 μmole/100 g for dexamphetamine. The effects on posture of dexamphetamine (3.0 μmoles/100 g) injected intraperitoneally are shown in Fig. 6.

The first sign, 2 to 5 min after injection, was drooping of the wings (Fig. 6, B and C) and increased flexion of all joints of the lower limb except the tarso-metatarsal. Consequently the bird walked with its trunk lower and more horizontal (Fig. 6, B and C). The drooping of the wings differed from that elicited by (±)-α-methylnoradrenaline in that the wings were held away from the trunk (compare Fig. 4, C with Fig. 6, C). At this stage, the amount of pecking was increased; the chicken was aggressive and if in the flock would even attack larger birds. Gait was unsteady and broad-based. Ultimately, with extreme flexion of the lower limbs the trunk rested on the floor (Fig. 6, D). Examination of the bird revealed increased muscle tone particularly in the leg flexors and sustained when the legs were passively displaced. With large doses, head retraction occurred, the neck and head being immobile; the tail was elevated by partial extension of the lower limbs (Fig. 6, D). Maximum postural changes were reached 10 to 30 min after injection and remained maximal for about 5 min. The bird could not move normally, however stimulated, nor balance nor perch, and if placed supine was unable to right itself.

During recovery, neck movements returned first so that the bird could look round. Next, further extension became possible in the lower limbs allowing waddling movements although the ventral surface of the trunk still brushed the floor. The range of extension

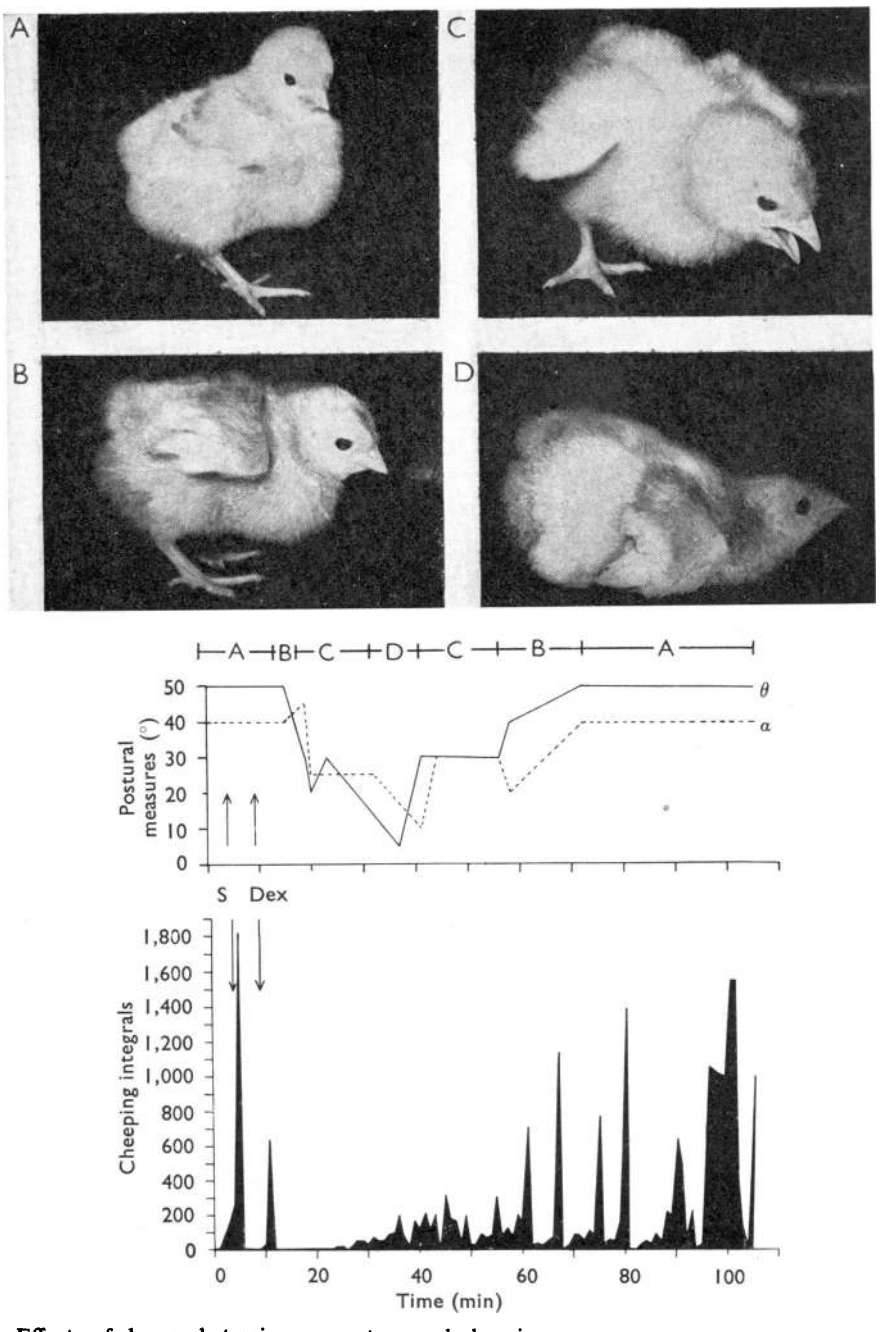

Fig. 6. Effects of dexamphetamine on posture and cheeping.

Photographs: 4-day-old chick, 40 g. A, control after saline injection; B, C and D, 2, 5 and 10 min, respectively, after 3.0 μmoles/100 g of dexamphetamine given intraperitoneally. Note increased leg flexion (B), wing extension, (C) head retraction and tail elevation (D).

Upper graph: postural measures (tarsal angle, α, and trunk rotation, θ). These angles decline 5 min after dexamphetamine (Dex, 3 μmoles/100 g, intraperitoneally) falling to nadir lasting for 10 to 20 min, then gradual return to normal; A, B, C and D above graph show corresponding postures (photographs) with changes in postural measure. Lower graph: cheeping absent or minimal whilst postural changes maximal, but return as postural changes abate. S=saline.

slowly increased in the lower limbs so that the bird could next elevate its body clear of the floor and walk, but the trunk remained horizontal and the wings drooped. Finally, normal posture returned some 40 to 70 min after injection.

The postural changes after dexamphetamine are plotted in terms of the trunk rotation angle and the tarsal angle in Fig. 6 (upper graph) which shows onset, offset, duration and intensity of action. In addition, Fig. 6 (lower graph) demonstrates that, while the postural changes are maximal, cheeping is absent and does not return until the postural effects abate.

Electromyograms were also recorded from the anterior and posterior thigh muscles during the development of postural changes. Dexamphetamine (1 μmole/100 g, intravenously) initially increased the potentials more in the flexors than in the extensors. With tail elevation, extensor activity, presumably compensatory to the effect of dexamphetamine on the leg flexors, increased considerably whereas flexor activity waned. α-Methylnoradrenaline (0.5 μmole/100 g, intravenously) diminished dexamphetamine-induced muscle potentials and this antagonism was more marked in limb extensors than flexors.

Cheeping and twittering. The excitant amines either increased both the number of cheeps and the duration and intensity of individual cheeps or produced twittering with its rapid high-pitched low intensity calls. The increased duration and intensity of cheeps produced by amphetamine is shown in Fig. 4, D of the previous paper (Dewhurst & Marley, 1965a).

These cheeping responses were not immediate. In the experiment of Fig. 7 five doses of dexamphetamine were injected intraperitoneally in randomized order at 24 hr intervals. All three doses with marked effects showed an initial period of about 10 min in which the bird was silent. Bursts of cheeping then alternated with silences lasting about 10 min. Twittering next developed so that calling became almost continuous and hence, even though intensity of call diminished, cheeping integrals increased. Respiratory rate rose to 120 per min; with twittering, the bird called throughout respiration. If marked postural changes developed, then all forms of cheeping were diminished or absent until the postural effects started to wane (Fig. 6, graphs). Consequently, although cheeping (with or without twittering) was increased by dexamphetamine when measured over the first 90 min, there was an optimal dose. This was 3.16 μmoles/100 g of dexamphetamine in the experiment of Fig. 7. The effects of larger doses on cheeping or twittering were proportionately less whilst postural effects were progressively greater. This is shown in the left-hand graph of Fig. 7, smaller cumulative integrals being obtained with the 3.56, 4.00 and 4.46 μmoles/100 g doses than with 3.16 μmoles/100 g of dexamphetamine, and also in the dose/response slope in the right-hand graph of Fig. 7. The progressively diminished cheeping could not be due to tachyphylaxis, since the postural effects also increased. Moreover, cheeping was enhanced or twittering developed as the postural effects abated, and the duration of this effect, measured in hours, was directly proportional to dose. Thus, changes in cheeping were sometimes secondary to other effects produced by the drug.

The effects of the excitant amines on posture, electrocortical and electromyographic activity were consistent and apparently depended only on drug factors, since they were regularly obtained whether the chicken was studied in isolation or in the presence of

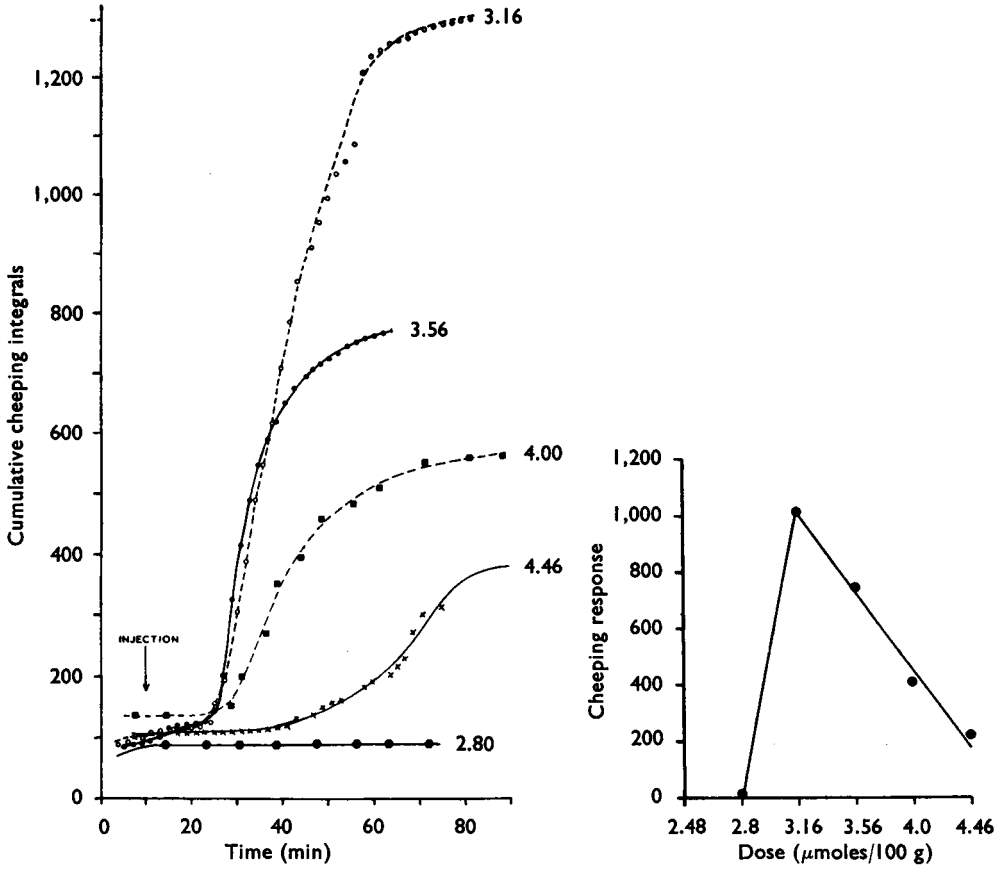

Fig. 7. Graphs of the effect of dexamphetamine on cheeping. Cumulative integrals of cheeping (left-hand graph) and plot of dose (log scale) against response (right-hand graph). 15-day-old chicken; 85 g. Doses of dexamphetamine (in μmoles/100 g, on right) were given intraperitoneally (at Injection) in random order at 24 hr intervals. Maximal increase in cheeping was with dexamphetamine, 3.16 μmoles/100 g; note the delay of 15 min after injection before cheeping increases. With increasing doses above 3.16 μmoles/100 g, cheeping is greater than control values but there is a progressively smaller response.

companions. The development of twittering, however, depended on extraneous stimuli as well. The dependence of twittering on social stimuli was apparent in two ways. Firstly, by its less frequent development in isolated animals when compared with chickens in a flock and, secondly, by its production in the isolated chicken on handling or introducing a companion. Specific social stimuli were necessary. A chicken twittering after dexamphetamine (1 μmole/100 g, intravenously) developed distress calls when its companion was removed. Brief twittering reverting to distress calls or occasional silence occurred when a companion animal was replaced by an inanimate object or another species. Gentle handling was more effective but only induced twittering in a minority. Sustained twittering in the majority of birds occurred only when a companion animal was present. To establish this finding more definitely, a crossover test was carried out with eighteen chickens. All were given dexamphetamine and half were left in a flock

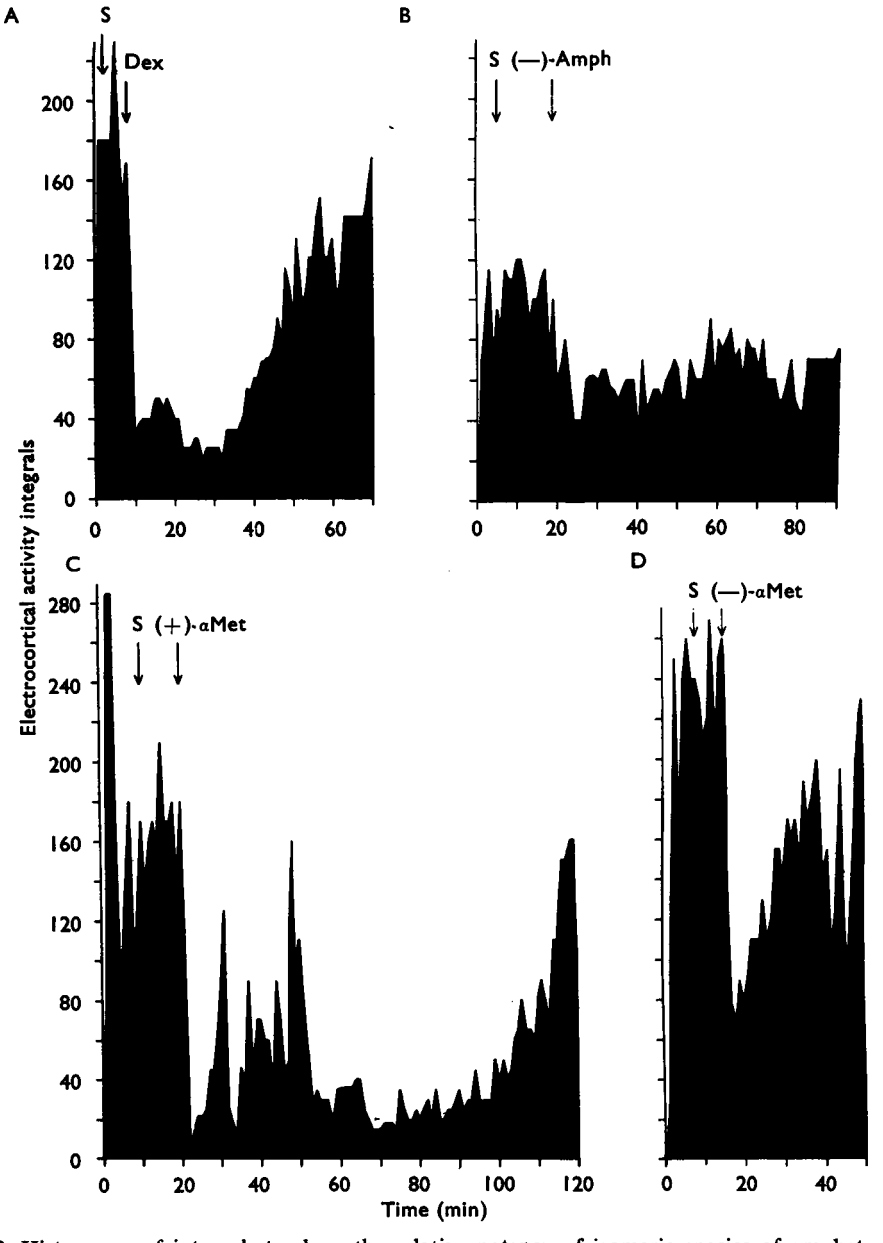

Fig. 8. Histograms of integrals to show the relative potency of isomeric species of amphetamine and α-methyltryptamine on electrocortical activity.

A: considerable fall in integrals of electrocortical activity associated with behavioural and electrocortical alerting produced by dexamphetamine (Dex, 2.0 μmoles/100 g, intravenously) in conscious unrestrained 8-day-old chicken; B: much smaller fall in integrals of electrocortical activity associated with less marked behavioural alerting in same chicken given (−)-amphetamine (−)-Amph, 2.0 μmoles/100 g, intravenously) 24 hr later.

C and D: much greater potency of (+)- than of (−)-α-methyltryptamine (0.5 μmoles/100 g, intravenously) in producing electrocortical alerting with fall in integrals of electrocortical activity. C: electrocortical alerting more intense and sustained with (+)-α-methyltryptamine ((+)-αMet) in conscious unrestrained 10-day-old chicken. D: record from same chicken 24 hr later but with (−)-α-methyltryptamine ((−)-αMet). S=saline, 0.2 ml. in all graphs.

and the others individually isolated. The following day all received dexamphetamine again and the birds previously isolated were left in a flock and the others kept solitary. Twittering occurred in seventeen of the eighteen when in the flock, whereas only two twittered in isolation. This difference is highly significant ($\chi^2 = 13$; d.f. $= 1$; $P<0.001$).

Twittering was obtained only in 1 to 28 day chickens at optimal temperature, and did not occur if the birds were cold, when distress calls occurred. Twittering was very occasionally heard in normal groups of chickens.

Stereoisomerism

Potency of both depressant and excitant amines depended in part on steric factors.

α-Methylnoradrenaline. This molecule has two asymmetric carbon atoms: isomerism specified here applies to the β-carbon atom. In tests with three chickens, racemic α-methylnoradrenaline was at least four times as potent in terms of threshold dose as the dextro-form.

Amphetamine and α-methyltryptamine. Optical activity here relates to the asymmetrical α-carbon atom. The dextroform was more active than the laevo-isomer for excitatory amines. Thus, in terms of threshold dose both dexamphetamine and (+)-α-methyltryptamine were four times as potent as the respective laevo-variety. Tests were made with the different isomers injected intravenously into the same chicken and 24 hr were allowed for recovery; the effects on electrocortical activity expressed as integrals are shown in Fig. 8. After (—)-amphetamine (2.0 μmoles/100 g) electrocortical alerting developed and, with the diminution in amplitude of electrocortical potentials, the electrocortical integrals declined from 100 to 40 within 4 min and then, as alerting subsided, gradually returned to preinjection values (Fig. 8, B). With dexamphetamine (2.0 μmoles/100 g) the electrocortical integrals fell from 170 to 33 at 2 min and subsequently dropped to 20, regaining preinjection values after 60 min (Fig. 8, A). Electrocortical arousal was therefore much more intense with the dextro-isomer. Postural effects were minimal (Grade 1) with (—)-amphetamine but extreme (Grade 3) with dexamphetamine. Cheeping was absent throughout both tests. However, on removing the bird from the sound-insulated box when the postural effects had abated, typical twittering developed with dexamphetamine but not with (—)-amphetamine. The fact that twittering developed was presumably due to handling and other stimuli (noise) in the laboratory, and that it developed with the dextro- but not the laevo-isomer of amphetamine indicated the former's greater potency. Similar differences in potency on electrocortical activity (Fig. 8, C and D), postures and cheeping were obtained with 0.5 μmole/100 g, intravenously of (+)- or (—)-α-methyltryptamine. Electrocortical alerting lasted 100 min and was more intense with (+)-α-methyltryptamine, compared to a duration of 40 min with the laevo-isomer. The results with the isomers of amphetamine and of α-methyltryptamine were confirmed in four other chickens.

Physiological antagonism

The central excitant and depressant amines were physiological antagonists to each other. Thus, a dose of (±)-α-methylnoradrenaline temporarily abolished the effects of an equimolar dose of dexamphetamine or of (+)-α-methyltryptamine on cheeping,

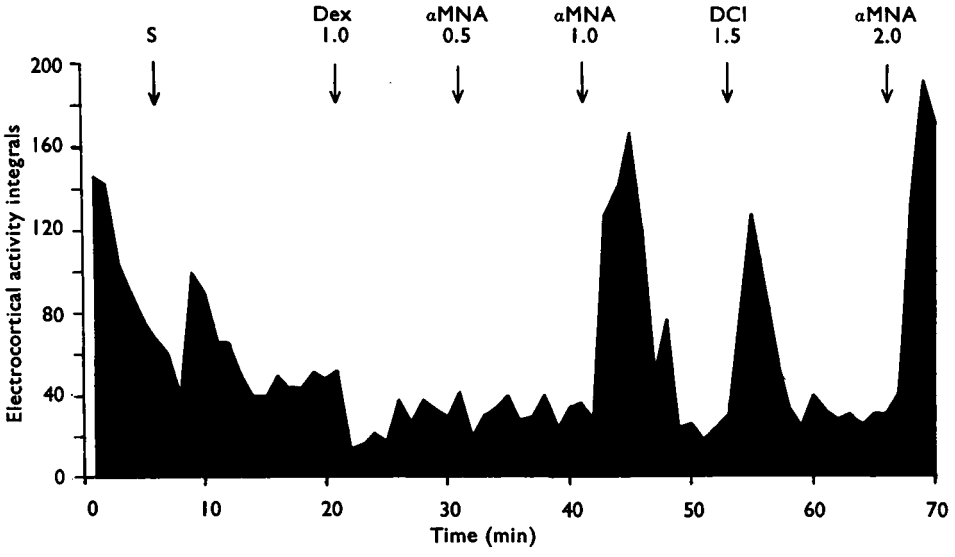

Fig. 9. Histogram of integrals to show the effects of dexamphetamine on electrocortical activity antagonized by (±)-α-methylnoradrenaline and dichloroisoprenaline. Conscious unrestrained 11-day-old chicken; 75 g. Electrocortical alerting (decreased integrals) produced by amphetamine (Dex, 1.0 μmole/100 g). α-Methylnoradrenaline (αMNA, 0.5 μmole/100 g) has no effect on electrocortical activity. Subsequently α-methylnoradrenaline (1.0 μmole/100 g) and dichloroisoprenaline (DCI, 1.5 μmole/100 g) temporarily antagonize the effects of amphetamine (note increased integrals for electrocortical activity). S=saline, 0.1 ml. All injections intravenous.

movement, posture, electrocortical and electromyographic activity, and produced sleep. The antagonistic effects on electrocortical activity are shown in Fig. 9. This tracing was taken from an alert chicken. The injection of dexamphetamine (1 μmole/100 g), led to further alerting as shown by the reduction in electrocortical integrals from about 50 to 25 per min. The intravenous injection of α-methylnoradrenaline (0.5 μmole /100 g) was ineffective but the injection of 1.0 μmole/100 g was followed immediately by sleep. This is shown in the tracing by the enormous increase in electrocortical integrals due to the development of large amplitude electrocortical activity. The electrocortical integrals rose from 25 per min to a peak of 150, 5 min after the injection. The effect was over in 10 min so the action of (±)-α-methylnoradrenaline after dexamphetamine was much shorter than in the untreated chicken in which it would have evoked sleep for 60 min or longer. Once the effect of α-methylnoradrenaline had abated, electrocortical alert activity returned. Subsequent intravenous doses of dichloroisoprenaline (1.5 μmole/100 g) and of (±)-α-methylnoradrenaline (2.0 μmole/100 g) evoked sleep accompanied by a large increase of electrocortical integrals. Thus, physiological antagonism was surmountable and reproducible.

The sleep produced by (±)-α-methylnoradrenaline was reversed briefly by the injections of equimolar doses of dexamphetamine or of (+)-α-methyltryptamine. The dextro-isomers were more potent as physiological antagonists than the laevo-isomers.

Pharmacological antagonism

Most potential antagonists have actions of their own on the central nervous system and

thus in certain doses may act as physiological antagonists. It was important to allow for such effects when assessing pharmacological antagonism. Hydergine in small doses produced electrocortical alerting; phenoxybenzamine and larger doses of Hydergine elicited long-lasting behavioural and electrocortical sleep. (±)-Pronethalol produced behavioural and electrocortical alerting. Methysergide, in intravenous doses of 0.001 to 0.1 μmole/100 g, affected neither behaviour nor cerebral electrical activity; larger doses were excitant. Consequently, in some experiments the agonist was tested and then retested after a small dose of antagonist; in others, large doses of antagonist were injected over several days and, when its central actions had abated, a normally effective dose of agonist was given. It is possible in this last group that efficacy of the antagonist waned as its agonistic effects disappeared although with phenoxybenzamine, which undergoes covalent bonding (Nickerson, 1949), this was unlikely. In experiments with methysergide a range from 0.001 to 1.0 μmole/100 g was tested against 1 μmole/100 g of agonist; with the other antagonists, a range from 0.1 to 1,000 μmole/100 g was tested against 1 μmole/100 g of agonist. Experiments with antagonists were made in forty-two chickens.

Antagonists at peripheral tryptamine receptors. Methysergide, a potent antagonist of the effects of tryptamine at peripheral receptors (Doepfner & Cerletti, 1958) antagonized the excitant effects of dexamphetamine and of (+)-α-methyltryptamine. In the first series of tests, methysergide was injected after the effects of the two amines on cheeping, posture, electrocortical and electromyographic activity had developed; these effects would normally have persisted for several hours with the doses chosen. Antagonism by methysergide began within 0.5 to 10 min and lasted from 20 min up to several hours; as antagonism waned, the excitant effects of the agonist returned, suggesting antagonism was surmountable. The antagonistic actions on cheeping and electrocortical activity were easier to obtain than on posture or the electromyogram. Antagonism by methysergide (0.001 μmole/100 g, intravenously) of the effect of (+)-α-methyltryptamine (0.5 μmole/100 g, intravenously) on electrocortical activity is shown in Fig. 10. The chicken was initially drowsy. Electrocortical alerting was produced by α-methyltryptamine as shown by the diminution of electrocortical integrals. Electrocortical alerting subsided in the ensuing 10 min after the injection of methysergide. The potency of methysergide was evident, for in a dose which by itself had no effect on electrocortical activity it antagonized the effects of a 500-times greater dose of agonist. Antagonism was probably competitive for it was surmountable, although this is not shown in the figure. An additional point illustrated in Fig. 10 is that, although a subsequent smaller dose of (+)-α-methyltryptamine, 0.125 μmole/100 g, did not surmount antagonism, 0.5 μmole/100 g of dexamphetamine did so. Onset of the excitant effects of dexamphetamine, after antagonism of α-methyltryptamine by methysergide, was immediate.

In the second series of tests, methysergide was injected first. The intravenous dose of methysergide (0.001 μmole/100 g) was without action on cheeping, electrocortical or electromyographic activity. In one such test, dexamphetamine (2 μmole/100 g) was injected 12 min later. Its action was diminished; instead of immediate and sustained electrocortical alerting with increase in cheeping after 10 min, alerting developed for 4 min only and the cheeping integrals rose slowly from 20 to 30 per min. The effects on electromyographic activity were unimpaired.

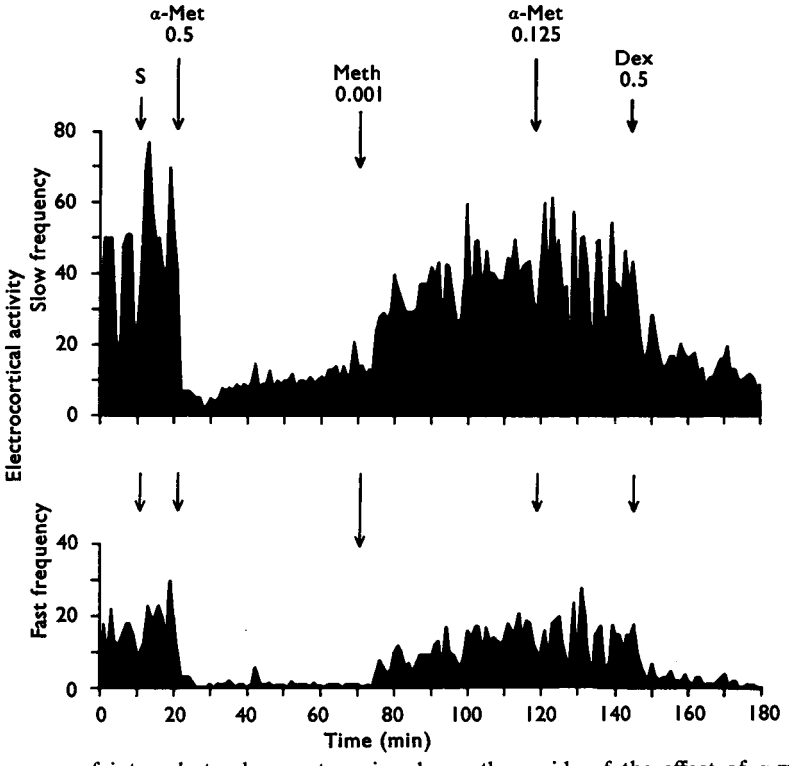

Fig. 10. Histograms of integrals to show antagonism by methysergide of the effect of α-methyltryptamine on electrocortical activity. 12-day-old chicken; 80 g. (+)-α-methyltryptamine (α-Met, 0.5 μmole/100 g) produced electrocortical alerting associated with a precipitate fall in the integrals of both fast and slow frequency electrocortical activity. The injection of methysergide (Meth, 0.001 μmole/100 g) antagonized the alerting produced by α-methyltryptamine and the integrals return to control values. A further but smaller dose of (+)-α-methyltryptamine (0.125 μmole/100 g) was ineffective but antagonism was surmounted by dexamphetamine (Dex, 0.5 μmole/100 g) which restored electrocortical alerting with reduction in integrals. S=saline. All injections intravenous.

Methysergide was, therefore, an excellent antagonist to the excitant amines. It did not antagonize the central depressant effects of (±)-α-methylnoradrenaline (0.1, 0.25, 0.5 and 1.0 μmole/100 g, intravenously).

Antagonists at peripheral sympathetic α-receptors. Phenoxybenzamine and Hydergine, potent antagonists of adrenaline at peripheral α-receptors (Goodman & Gilman, 1955), were used. Large doses of phenoxybenzamine were given intraperitoneally over a number of days as single small doses given intravenously proved ineffective. Phenoxybenzamine was only partially effective against (±)-α-methylnoradrenaline and ineffective against dexamphetamine and (+)-α-methyltryptamine. Hydergine did not antagonize the effects of any of the three amines tested.

Thus (±)-α-methylnoradrenaline, 0.5 and 1.0 μmole/100 g, intravenously, doses which would normally have put the chicken to sleep for 10 to 90 min, was ineffective after treating the bird with enormous doses of phenoxybenzamine (706 μmole/100 g, injected intraperitoneally in divided doses over 4 days). Larger intravenous doses of α-methyl-

noradrenaline (2.0, 8.0 and 16 μmoles/100 g) were given in an attempt to surmount antagonism but these elicited behavioural and electrocortical alerting. The lack of depressant effect of α-methylnoradrenaline was not due to loss of activity, for 0.5 and 1.0 μmole/100 g of the same solution given intravenously to two control chickens of similar age produced sleep. The depressant action of α-methylnoradrenaline wanes as the chicken matures. However, the lack of depressant effect was not due in this instance to the maturity of the chicken for, 7 days later, α-methylnoradrenaline, 0.5 and 1.0 μmole/100 g, intravenously, previously ineffective, now elicited sleep. The depressant action of α-methylnoradrenaline (1.0 μmole/100 g, intravenously) was antagonized by phenoxybenzamine (100 μmoles/100 g, intraperitoneally given over 2 days) in a further chicken, but not in a chicken given a smaller intraperitoneal dose of phenoxybenzamine (10 μmoles/100 g, given over 2 days). The effects of dexamphetamine (2.0 μmoles/100 g, intravenously) and (+)-α-methyltryptamine (2.0 μmoles/100 g, intravenously) appeared to be unaltered by phenoxybenzamine (2 μmoles/100 g, intravenously, and 7.35 and 26 μmoles/100 g, intraperitoneally, respectively injected over 2 days) in three experiments.

The effects of dexamphetamine (1.0 μmole/100 g), of (+)-α-methyltryptamine (0.5 μmole/100 g) and (±)-α-methylnoradrenaline (0.5 μmole/100 g) were unaltered by Hydergine (0.002, 1.0 or 2.0 μmole/100 g); all these injections were intravenous.

Antagonists of peripheral sympathetic β-receptors. Dichloroisoprenaline and pronethalol, which block peripheral β-receptors (Powell & Slater, 1958; Black & Stephenson, 1962), behaved also as agonists. Dichloroisoprenaline (1.5 μmole/100 g, intravenously) produced behavioural and electrocortical sleep. As shown in Fig. 9 it behaved as a *physiological* antagonist to dexamphetamine, evoking sleep with large amplitude electrocortical potentials, the electrocortical integrals increasing from 30 to 130 per min. When these effects had subsided after about 7 min, this dose of dichloroisoprenaline did not prevent the depressant effects of (±)-α-methylnoradrenaline (2.0 μmoles/100 g, intravenously) on electrocortical activity when electrocortical integrals rose from 30 to 190 per min. In another test, dichloroisoprenaline (1 μmole/100 g) did not prevent the effects of dexamphetamine (1 μmole/100 g) on cheeping (integrals rising from 30 to 60 per min) or electrocortical activity (integrals falling from 65 to 20 per min), or the effects of (±)-α-methylnoradrenaline (1 μmole/100 g). Pronethalol in doses smaller than 10 μmoles/100 g did not antagonize the effects of equimolar doses of α-methylnoradrenaline. Pronethalol given alone in doses greater than 10 μmoles/100 g had slight excitant effects. In such doses weak antagonism to the depressant effects of α-methylnoradrenaline occurred, presumably due to its agonistic excitant properties, that is probably physiological rather than specific antagonism.

Antagonists at acetylcholine or histamine receptors. The effects of (±)-α-methylnoradrenaline (1 μmole/100 g), dexamphetamine (1 μmole/100 g) and (+)-α-methyltryptamine (1 μmole/100 g) were not impaired by hyoscine (2 μmole/100 g) nor by the histamine antagonist, chlorpheniramine (2 μmole/100 g); all these injections were intravenous.

Substances affecting the noradrenaline store

Cocaine. A chicken given enormous intraperitoneal doses of cocaine (80 μmoles/100 g) on each of two successive days, showed only slight electrocortical arousal after dexamphetamine (2 μmoles/100 g, intravenously), a dose that usually would have produced

intense electrocortical alerting and extreme postural changes. However, a further similar dose of dexamphetamine given 1 hr later produced marked electrocortical alerting and postural changes (Grade 3). The effect of amphetamine was therefore diminished. (±)-α-methylnoradrenaline (2 μmoles/100 g) subsequently injected intravenously produced marked central depressant effects.

Reserpine. A chicken given two intraperitoneal doses of reserpine in 3 days (1 μmole/100 g) became drowsy and inactive and was difficult to alert with sensory stimuli. After dexamphetamine (2 μmoles/100 g, intravenously) there was immediate electrocortical alerting, the electrocortical integrals dropping from 340 to 160; marked postural changes (Grade 3) developed. This picture was sustained for 15 min when (±)-α-methylnoradrenaline (2 μmoles/100 g) was injected intravenously, which diminished the ataxic and postural changes due to amphetamine; 10 min later a second dose of (±)-α-methylnoradrenaline (2 μmoles/100 g) put the bird to sleep. These results were confirmed in three other chickens. The excitant effects of amphetamine and the antagonistic action of α-methylnoradrenaline were, therefore, apparently unimpaired.

Lethal effects of the amines

The excitant amines, (+)-α-methyltryptamine (two of twelve experiments) and dexamphetamine (eleven of some 250 experiments) in doses within the customary range, produced sudden death after intravenous injection; on rare occasions intraperitoneal injection was lethal in young and adult chickens. This was not observed with α-methylnoradrenaline given intravenously or intraperitoneally.

DISCUSSION

Young chickens were chosen for these experiments because immature animals have imperfect or non-existent blood-brain barriers (Bakay, 1956; Lajtha, 1957). Consequently injected substances such as the catechol amines would not be prevented from reaching central neurones, as they would when a blood-brain barrier is present. Comparison of the responses of the young and the mature animal to the same drug should indicate the influence of the blood-brain barrier in determining central effects of the amines.

Waelsch (1955) found that adult blood-brain barrier characteristics only developed in the chicken at about the fourth week of life. It seems significant, therefore, that the depressant response to catechol amines wanes just at this time and the excitatory response typical of the adult animal supervenes (Key & Marley, 1962). Further, catechol amines cease to act as physiological antagonists to amphetamine-like amines in the chicken *encéphale isolé* preparation at the fourth week (Marley, 1963). A factor such as maturation of the blood-brain barrier or perhaps enzyme changes preventing central depressant activity is strongly suggested. It is supported also by results in other species; Waelsch (1955) found that the guinea-pig has a mature blood-brain barrier when newly born, and catechol amines elicited behavioural and electrocortical alerting from this time until adulthood (Marley & Key, 1963).

A second reason for using the chick was that three groups of amines could be distinguished by the behavioural and electrocortical responses to them (Key & Marley, 1962). In most animals apparent central effects of sympathomimetic amines are qualita-

tively similar ; the differentiation found in the chick permitted study of structure-activity relationships. These would have validity irrespective of the underlying mechanisms ; as similar groupings have been observed in other species (Fleckenstein & Burn, 1953 ; Vane, 1960 ; Marley, 1962) they appear to have a general biological significance.

These earlier findings in the chicken, including the observation that α-methyltryptamine had similar effects to amphetamine (Dewhurst & Marley, 1964), were confirmed and considerably amplified in the present experiments. By using the methods developed for quantification it was clear that response to the central depressant and excitant amines consisted of total patterns of responses with different durations and thresholds. The difference in durations of response of the various modalities showed up particularly with the larger doses of the amines.

The reaction to α-methylnoradrenaline comprised diminution or abolition of vocalization, of movement and of electromyographic activity, with the development of drowsiness or sleep accompanied by large amplitude electrocortical potentials. These effects were allied to those observed during physiological sleep rather than to those observed during anaesthesia, since the chicken was rousable by sensory stimuli and postural activity was retained. The posture assumed during sleep produced by α-methylnoradrenaline was the antithesis of that observed after amphetamine or α-methyltryptamine. With α-methylnoradrenaline, the neck and head drooped and there was diminution of muscle tone. With the excitant amines, there was neck retraction, enhanced muscle tone and elevation of the tail.

Of the effects produced by amphetamine and α-methyltryptamine the most consistent were the electrocortical alerting and increased electromyographic potentials which preceded any increase in the amount of movement or cheeping. In fact if cheeping was increased then the bird moved very little. This inverse relation between cheeping and movement had been noted for normal activity (Dewhurst & Marley, 1965a). It was also noted in the operant situation when the bird had been trained to peck a lighted disc for food. When the chicken was pecking at a high rate, cheeping was absent or minimal ; when pecking had ceased or was sporadic, there was considerable cheeping (Marley & Morse, 1965).

These observations of normal behaviour have much relevance for interpreting behavioural changes produced by drugs. An apparently marked action on behaviour may be subsidiary to some other less conspicuous action of the drug or to a combination of drug with non-drug factors. This was shown very clearly with twittering which developed readily in groups of chickens but less readily in solitary chickens given excitant amines and was reminiscent of the observations by Gunn & Gurd (1940) that " the presence of other mice induced excitation in mice receiving high doses of amphetamine," and those of Chance (1946, 1947) that sound and aggregation profoundly affected amphetamine-toxicity in mice.

The effects on posture also developed after a delay, contrasting with their rapid onset after α-methylnoradrenaline. The postural changes affected all behavioural patterns, so that, whilst they were most intense, cheeping and movement were eclipsed and only reappeared when the postural effects abated.

No satisfactory pharmacological antagonist was found to the central depressant effects of (±)-α-methylnoradrenaline. Phenoxybenzamine showed some antagonism but large doses were required. However, the actions of the β-haloalkylamines in the chicken may be atypical, for Harvey & Nickerson (1951) observed that surprisingly large doses of dibenamine were required to abolish the pressor action of adrenaline. The results suggest that an antagonist at peripheral sympathetic α-receptors was more likely to be effective than one at β-receptors.

Because of the similar effects of α-methyltryptamine and amphetamine and the abolition of their actions by methysergide and not by other pharmacological antagonists, it is likely that these amines produce their central effect by an action on " tryptamine receptors " in the brain. The findings support the suggestion by Vane (1960) and Gelder & Vane (1962) that amphetamine may act on central tryptamine receptors.

Amines such as amphetamine act indirectly on peripheral tissues such as blood vessels, by causing noradrenaline release (Burn, 1960). However, a number of reasons suggest that the central excitant effects of amphetamine cannot be ascribed in the chicken to intracerebral noradrenaline release. First, the excitant effects obtained after treating the chicken with reserpine which depletes the tissues of noradrenaline (Holzbauer & Vogt, 1956 ; Burn & Rand, 1957) or after treatment with cocaine, which competes for the noradrenaline store (Farrant, 1963). Moreover, because noradrenaline was a central depressant in the young chicken, the major action of the excitant amphetamine-like amines was unlikely to be due to release of noradrenaline in the brain (Key & Marley, 1962). This is not to say that noradrenaline release does not occur, since both amines are present in the brain of the young chicken (0.39 μg of noradrenaline and 0.11 μg of adrenaline per g of whole brain ; Borowitz & Marley, unpublished) and amphetamine has been shown to diminish the brain noradrenaline concentration in rabbits (Sañan & Vogt, 1962) and rats (McLean & McCartney, 1961). If, in the chicken, intracerebral noradrenaline release did occur, the effects may have been obscured by the predominant tryptamine-like effects of amphetamine.

Points such as these will require further clarification. Nevertheless, the results so far obtained allow the two types of amine to be clearly distinguished in terms both of their effects and of their pharmacological antagonists. These means of distinction will serve as a model in considering the action of an extensive series of amines to be reported in a subsequent paper (Dewhurst & Marley, 1965b).

SUMMARY

1. The effects of the α-methyl derivatives of noradrenaline (Cobefrine) of phenylethylamine (amphetamine), and of tryptamine (α-methyltryptamine) were measured on behaviour, electrocortical and electromyographic activity in young chickens under controlled conditions.

2. (±)-α-Methylnoradrenaline produced all the features of sleep, namely, a sleeping posture (standing or squatting) with closed eyes, diminished or absent cheeping and movement. These were associated with large amplitude slow (1 to 4 cycles/sec) electrocortical potentials and diminished electromyographic activity. The chicken could be roused by sensory stimuli. With the larger doses, muscle hypotonia and ataxia were

marked. The various components of the total sleep pattern had differing thresholds and durations. The total sleep response was only present in the first 3 to 4 weeks of life and was not demonstrable after 2 months.

3. Dexamphetamine and (+)-α-methyltryptamine produced the features characteristic of the fully alert animal, namely, increased movement and vocalization, and increased sensitivity to environmental stimuli. These were associated with small amplitude, fast (10 to 30 cycles/sec) electrocortical potentials and increased electromyographic activity. With large doses characteristic postural changes occurred comprising wing extension, head retraction, and ultimately, the bird was immobile with its chest on the ground and its tail elevated. If placed supine righting did not occur. The effects on electrocortical and electromyographic activity were immediate; those on cheeping and posture only became obvious after a delay. The increased vocalization comprised an increase in ordinary cheeping or a characteristic "twittering." The development of twittering was dependent on social stimuli as well as excitant amines for it was evoked easily in birds with companions but rarely in isolated animals. All forms of vocalization were diminished or absent if postural changes were marked, but returned as posture recovered. The alerting responses showed no change as the animal matured.

4. Optical activity affected potency. Thus racemic α-methylnoradrenaline was more active than the dextro-isomer. In contrast dextro-isomers were more active than the laevo-forms of the excitants amphetamine and α-methyltryptamine.

5. Physiological antagonism occurred between depressant and excitant amines on cheeping, movement and posture, and on electrocortical and electromyographic activity.

6. Specific antagonists of tryptamine (for example methysergide) were potent antagonists of the central excitant effects of amphetamine and of α-methyltryptamine. Specific antagonists of peripheral sympathetic α-receptors, (for example phenoxybenzamine) showed only slight antagonism to the central depressant effects of α-methylnoradrenaline. Specific antagonists of peripheral sympathetic β-receptors, acetycholine receptors, and histamine receptors, were ineffective against either depressant or excitant amines.

7. Treatment for 3 days with reserpine did not affect the responses to amphetamine or α-methylnoradrenaline, but an enormous dose of cocaine caused slight impairment of the effects of amphetamine.

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