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ACTION OF SYMPATHOMIMETIC AND ALLIED AMINES ON THE CENTRAL NERVOUS SYSTEM OF THE CHICKEN

BY

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The effects of the α-methyl derivatives of noradrenaline, phenethylamine and tryptamine on the central nervous system of the chicken have been described (Dewhurst & Marley, 1965a). In the present experiments a larger series of amines and of their amino acid precursors has been tested on the central nervous system and on blood pressure. In addition, factors potentiating activity such as monoamine oxidase inhibition or diminishing activity such as tachyphylaxis or pharmacological antagonism have been studied.

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

METHODS

The methods used for implanting electrodes and cannulae and for measuring cheeping and electrocortical and electromyographic activity were the same as those described previously (Dewhurst & Marley, 1965a,b). In addition to 1- to 28-day-old chickens, fowls up to 1 year old were used.

To record blood pressure in the conscious unrestrained chicken a polyethylene cannula sealed with a nylon spigot and containing heparin-saline (100 mg/ml.) was tied into the upper part of a carotid artery; the tubing was brought through the scalp incision and fixed to the skull and wound into the strand of electrodes and the venous cannula. In the experiments in which electrocortical activity and blood pressure were recorded, the cortical electrodes were implanted on the left cerebral hemisphere and the blood pressure cannula was tied into the right carotid artery. At the time of the experiment, the spigot was removed and the arterial catheter was connected via saline-filled polyethylene tubing to a tap unit and screening micrometer transducer. The signals were amplified and displayed on a D.E. potentiometric recorder (Cambridge Instruments).

Other blood pressure experiments were made with spinal chickens. These were prepared with halothane and oxygen anaesthesia given by endotracheal tube. After destroying the brain through an occipital craniotomy, the animal was artificially ventilated with a Palmer Miniature Ideal respiration pump. Excess air was allowed to escape through an adjustable leak in the circuit and round the endotracheal cannula. Blood pressure was recorded with a transducer connected to a cannula in the carotid or the ischiadic artery.

Chromatography was carried out with the apparatus and techniques devised by Smith (1960). Amines were applied as the salts (detailed below) dissolved in 50% methanol in water to give an amine concentration of 10 μmoles/ml. Spots (1 μl.) were applied with a Hamilton syringe. The major component of the developing solvent was oleyl alcohol (B.D.H.) which had been washed successively with equal volumes of N-sodium hydroxide, de-ionized water, N-hydrochloric acid, further water until washings were neutral and then shaken with an equal volume of 0.1 N-ammonium acetate buffer, *p*H 7.4, for 20 min. The two

phases were allowed to separate by standing, but kept in the same container. The mobile phase for development was made with five parts of the oleyl alcohol phase, one part of the ammonium acetate phase, and four parts of ethanol (all by volume). The chamber was lined with sheets of Whatman paper soaked in the solvent mixture. Chromatograms were allowed to develop for 15 hr at room temperature. The ethanol and water were allowed to evaporate from the chromatograms and the amines were located first by ultraviolet light (254 and 360 mμ), then by 0.5% ninhydrin in ethanol and finally by 0.5% 2,6-dichlorobenzoquinone-4-*N*-chloroimine in ethanol.

Definitions of twittering and grades of postural changes are given in the previous paper (Dewhurst & Marley, 1965a). Optimal dose is defined as a dose greater than the threshold dose, which produces clear-cut reversible effects of at least 4 to 5 min duration on both cheeping and electrocortical activity, thus allowing subsequent doses to be tested during the same experiment.

Drugs. These included the hydrochlorides of (+)-α-methylnoradrenaline[(+)-2-amino-1-(3,4-dihydroxyphenyl)propan-1-ol; (+)-Cobefrine], (±)-α-methylnoradrenaline[(±)-Cobefrine], cocaine, (±)-cyclopentamine, (−)-dichloroisoprenaline, dopa (L-3,4-dihydroxyphenylalanine), dopamine [4-(2-aminoethyl)-pyrocatechol], Epinine [4-(2-methylaminoethyl)pyrocatechol], (±)-α-ethylnoradrenaline, (±)-hydroxyphenethylamine, mescaline, (±)-metanephrine, (±)-methoxamine, *m*-methoxybenzylamine, *m*-methoxyphenethylamine, 3-(*m*-methoxyphenyl)propylamine, (+)-*m*-methoxy-α-methylphenethylamine, mebanazine-[(±)-α-methylbenzylhydrazine], (+)- and (−)-α-methyltryptamine, (±)-6-chloro-α-methyltryptamine, (±)-6-methoxy-α-methyltryptamine, (±)-α-6-dimethyltryptamine, (+)-noradrenaline, pargyline, phenoxybenzamine, (−)-phenylephrine, phenethylamine, (±)-phenylpropanolamine, pronethalol, propranolol, 1,2,3,4-tetrahydronaphthylamine, tryptamine, metatyramine and paratyramine; the hydrobromides of (±) - α - methyldopamine[4 - (2 - aminopropyl)pyrocatechol], (±) - hydroxyamphetamine[4 - (2 - amino - propyl)phenol], hyoscine; the sulphates of (+)- and (−)-amphetamine, (−)-isoprenaline, (±)-pholedrine, (±)-tranylcypromine and (±)-tuaminoheptane. Also tested were (−)-adrenaline and (−)-noradrenaline hydrogen tartrate, catechol (pyrocatechol), chlorpheniramine dimaleate, dihydroergotamine tartrate, Hydergine (the methanesulphonate of the dihydro-derivative of ergotoxine), 5-hydroxytryptamine creatinine sulphate, 6-hydroxytryptamine creatinine sulphate, (±)-metaraminol bitartrate, methysergide, (−)-noradrenaline bitartrate monohydrate, (±)-oxedrine tartrate, reserpine and L-tryptophan. Doses are expressed as μmoles per 0.1 kg body weight in the young chicken and per 1.0 kg body weight in the adult. An arbitrary molecular weight of 500 was given to Hydergine. All injections were intravenous unless otherwise stated.

RESULTS

Structure-activity relationships measured on cheeping and electrocortical activity

For all groups of amines, structure-activity relationships are deduced from relative potencies given in Table 1. The threshold and optimal doses are given, as are durations of actions of the amines and the number of chickens tested. Potency has been compared in terms of threshold dose, whereas duration of action is better represented by the optimal dose.

Central depressant amines

These amines produced physiological sleep with diminution or loss of cheeping and movement, a characteristic posture with retention of postural reflexes, large-amplitude slow-frequency electrocortical potentials and diminution or loss of electromyographic activity. These changes have been described in detail for α-methylnoradrenaline in a previous paper (Dewhurst & Marley, 1965a). The most potent depressant was (−)-adrenaline which produced effects in intravenous doses of 0.0025 μmole/100 g.

Variations in ethylamine side-chain. (1) N-*substituents.* Although *N*-methylation of (−)-noradrenaline to form (−)-adrenaline doubled depressant activity on cheeping and on the electrocorticogram, *N*-methylation had a reverse effect in the absence of the hydroxyl

on the β-carbon atom, dopamine being considerably more potent than Epinine. Substitution with the larger isopropyl-group also diminished potency, (−)-isoprenaline being 1/20th as active as (−)-adrenaline on cheeping and 1/400th as active on the electrocorticogram. Potency waned in the order (−)-adrenaline, (−)-noradrenaline, (−)-isoprenaline and (−)-phenylephrine which accords partly with Ahlquist's (1948) views of an action on α-receptors. (2) *α-Carbon substituents.* The mean threshold dose of (±)-α-methylnoradrenaline was five times greater than that of (−)-noradrenaline on cheeping and on the electrocorticogram; thus, α-methylation diminished potency. Duration of action was, however, increased, for with ten to twenty times the threshold doses the effect of α-methylnoradrenaline lasted for at least 90 min compared with 10 min for noradrenaline. In general, other substituents on the α-carbon also diminished potency; (±)-ethylnoradrenaline was 1/20th as active as (−)-noradrenaline and α-methylation of dopamine rendered it inactive. (3) *β-Hydroxylation.* The importance of the β-hydroxyl group was evident from the greater potency of adrenaline and noradrenaline compared to Epinine and dopamine respectively. Thus (−)-adrenaline was 400 times more active than Epinine on cheeping and the electrocorticogram. (−)-Noradrenaline was six times more potent than dopamine on cheeping and twenty times more active on the electrocorticogram. (4) *Stereoisomerism.* As a central depressant (−)-noradrenaline was twice as active as (+)-noradrenaline on the electrocorticogram and 100 times as active on cheeping. Racemic α-methylnoradrenaline (with respect to the β-carbon atom) was forty times as potent as the dextro-form. (5) *Variations in chain length.* Absence of the ethylamine side-chain considerably modified activity. The effect of catechol was compared with that of (−)-noradrenaline in two experiments. Whereas (−)-noradrenaline (0.01 μmole/100 g) elicited drowsiness and electrocortical slow wave activity and abolished cheeping, catechol (0.03 μmole/100 g) produced behavioural and electrocortical alerting.

Ring substitution. (1) *Phenolic groups.* Amines with a hydroxyl group substituted in the 3 or 4 position on the ring (meta- and paratyramine) were much less active than dopamine with hydroxyl groups in the 3 and 4 positions but equiactive or slightly less active than Epinine. (2) *Methoxy- and chloro-substituents.* Substances substituted in the ring with methoxy- (mescaline, metanephrine) or chloro-substituents (dichloroisoprenaline) showed less central depressant activity than the parent compound. Of these, mescaline was far the most potent, being one-fourth as active as (−)-adrenaline on electrocortical activity and one-fortieth as active on cheeping. 3-Methoxylation of adrenaline to form metanephrine diminished potency 800-fold both on cheeping and on the electrocorticogram.

Paradoxical effects. With larger doses (for example 0.1 μmole/100 g of (−)-adrenaline) all depressant amines elicited behavioural and electrocortical drowsiness interspersed with alert electrocortical activity. With even higher doses (for example (−)-adrenaline, 0.3 μmole/100 g) the response was one of electrocortical alerting but with behavioural drowsiness. As will be discussed later, these paradoxical effects may be related to changes in blood pressure produced by the higher doses.

Intermediate group

Responses to these amines appeared to have no consistent pattern. With the exception of (±)-α-ethylnoradrenaline and α-methyldopamine, the substances all possessed a single

TABLE 1

MEAN THRESHOLD DOSES AND OPTIMAL DOSES FOR EFFECTS ON CHEEPING AND ELECTROCORTICAL ACTIVITY (ECoG) TOGETHER WITH AVERAGE DURATION OF RESPONSE TO THRESHOLD AND OPTIMAL DOSES

Within each of the three main groups the most potent amine heads the list which is in descending order of potency. Results derived from chromatography of the amines are given on the right side of the table and related to the biological effects of the amines. For the R_Fs the number of determinations are in parentheses. * The partition coefficient for this compound was determined empirically in order to calculate the constant A_s/A_m for the system

		Cheeping				Electrocortical activity					
		Threshold dose		Optimal dose		Threshold dose		Optimal dose		Chromatography	
Action	No. of expts.	(μmole/100 g)	Duration (min)	(μmole/100 g)	Duration (min)	Drug	(μmole/100 g)	Duration (min)	(μmole/100 g)	Duration (min)	Mean R_F	Partition coefficient
Depressant	36	0·0025	8+	0·01	10	(−)-Adrenaline	0·0025	6	0·01	10	0·12 (4)	0·26
	42	0·005	6	0·02	10	(−)-Noradrenaline	0·005	5	0·02	10	0·10 (4)	0·21
	94	0·025	5	0·5	90+	(±)-α-Methylnoradrenaline	0·025	4	0·5	90+	0·12 (4)	0·26
	12	0·03	9+	0·1	10	Dopamine	0·1	2+	0·2	10+	0·10 (4)	0·21
	15	0·05	13+	0·1	30	(−)-Isoprenaline	1·0	6	1·5	30	0·11 (2)	0·24
	9	0·1	4	0·5	8	Mescaline	0·01	5	0·5	8	0·21 (1)	0·51
	6	0·5	4	0·5	4	(+)-Noradrenaline	0·01	3	0·5	4	0·09 (1)	0·19
	6	1·0	4+	2·0	6	Epinine	1·0	4	2·0	6	0·11 (2)	0·24
	3	1·0	2	5·0	5	(+)-α-Methylnoradrenaline	1·0	2	5·0	5		
	18	1·0	1	5·0	5	Paratyramine	1·0	2·5	5·0	5	0·10 (4)	0·21
	6	1·0	8+	2·0	30	(−)-Dichloroisoprenaline	1·5	11+	2·0	30	0·19 (1)	0·46
	3	2·0	5	2·0	5	(±)-Metanephrine	2·0	8	2·0	8	0·13 (2)	0·29
	3	2·5	—	2·5	3	Metatyramine	2·5	3	2·5	3		
	15	2·0	4	2·0	4	(−)-Phenylephrine	2·0	4	2·0	4	0·18 (1)	0·42
Depressant (low dose)	6	0·02	4+			(±)-6-Chloro-α-methyltryptamine	0·02	7			0·40 (1)	1·30
		0·1	4				0·1	4				
Excitant (high doses)	9	0·1	10			Metaraminol	0·1	7				
		10	22				10	27				
	9	0·1	3			(±)-α-Ethylnoradrenaline	0·1	3				
		0·5	29				0·5	29				
	6	1·0	10			(±)-6-Methoxy-α-methyltryptamine	1·0	10+			0·22 (1)	0·55
		2·0	10				2·0	9				

TABLE 1—*Continued.*

Inactive						Hydroxyamphetamine						
						m-Methoxybenzylamine						
						3-(*m*-Methoxyphenyl)-propanolamine						
						α-Methyldopamine					0·23 (2)	0·57
						(±)-Pholedrine						
Excitant	6	0·01	4 : 4	0·5	10 : 10	5-Hydroxytryptamine	0·01	4 : 4	0·5	10 : 10	0·11 (4)	0·24
Depressant		0·1	3 : 4+	0·1	3 : 4+	(±)-Oxedrine	0·1	3 : 4+	0·1	3 : 4+		
(at same dose)	6	0·2	0·5 : 5	2·0	10 : 10	6-Hydroxytryptamine	0·2	0·5 : 5	2·0	10 : 10	0·10 (2)	0·21
Excitant	42	0·001	3	0·01	10	Tryptamine	0·001	2	0·1	10	0·26 (16)	0·7
	33	0·01	4	0·05	10	Phenethylamine	0·01	6	0·5	8	0·34 (2)	1·0*
	6	0·1	3·5	0·2	8	(±)-Hydroxyphenethyl-amine	0·1	5+	0·5	8		
	6	0·12	30	0·5	360	(+)-α-Methyltryptamine	0·12	30	0·5	60	0·34 (3)	1·0
	200	0·5	30	1·5	120	Dexamphetamine	0·5	30	1·5	70	0·39 (1)	1·2
	3	—	—	—	—	(−)-α-Methyltryptamine	0·5	0	4·0	50		
	3	1·0	6	2·0	12	Tetrahydronaphthylamine	1·0	30	3·0	40+		
	6	—	—	—	—	(+)-*m*-Methoxy-α-methyl-phenethylamine	1·0	10	3·0	70		
	9	1·0	4	1·0	4	*m*-Methoxyphenethylamine	1·0	4	1·0	4		
	6	1·0	3	1·0	3	(±)-Phenylpropanolamine	1·0	6	1·0	6		
	9	—	—	—	—	(−)-Amphetamine	2·0	10+	4·0	30		
	6	1·0	20	1·0	20	(±)-Cyclopentamine	5·0	6+	10·0	30		
	9	2·0	15	2·0	15	(±)-Methoxamine	0·5	10	2·0	20	0·27 (1)	0·72
	14	5·0	10+	5·0	10+	(±)-Tuamine	5·0	5	10·0	25		

methoxy-, hydroxy- or chloro-substituent on the phenyl or indole ring. Seven of the twelve amines also had a substituent on the α-carbon atom which is associated with diminished potency.

The earlier view that this group consisted of amines with equivocal or inconsistent effects (Key & Marley, 1962) requires modification and further study has shown that there are three distinct sub-groups.

The first sub-group consisted of metaraminol, (±)-α-ethylnoradrenaline, 6-chloro-α-methyltryptamine and 6-methoxy-α-methyltryptamine which were central depressants in small doses but excitants at higher doses. The threshold dose for the excitant effect was one- to two-hundred times the threshold for the depressant effect.

The second sub-group consisted of five substances which had no detectable effect.

The third sub-group consisted of 5- and 6-hydroxytryptamine and oxedrine. They showed a biphasic action after a single dose; there was initial behavioural and electrocortical alerting followed after about 5 min by a typical central depression. Among other differences from the first sub-group the alerting component with 5- or 6-hydroxytryptamine was accompanied by a fall rather than a rise in blood pressure. Furthermore the alerting phase could be abolished by methysergide, a specific antagonist for the excitant amines. These substances, therefore, were presumably capable of activating both central depressant and central excitant receptors.

Central excitant amines

These amines produced alerting with increase in the amount of cheeping and movement, electrocortical alerting and augmented electromyographic potentials. In addition, those amines with long-lasting effects produced twittering and characteristic postural changes. These changes have been described in detail for α-methyltryptamine and amphetamine in the previous paper (Dewhurst & Marley, 1965a).

The cardinal features for excitant activity appeared to be ethylamine substituted in the β-position with one of a number of structures having the common attributes of lipid solubility allied to lack of electronegative substituents; the most potent excitants were tryptamine and phenethylamine.

Variations in ethylamine side-chain. (1) *α-Carbon substituents.* A methyl group on the α-carbon atom enhanced longevity of action. Thus equimolar doses (1.0 μmole/100 g) of phenethylamine and dexamphetamine had excitant effects for 10 and 60 min respectively; similar differences in duration of activity were found with tryptamine and (+)-α-methyltryptamine. As with the depressant substances, the α-carbon substituent also diminished potency. Thus, tryptamine and phenethylamine were about 100- and 50-times respectively more active than α-methyltryptamine and dexamphetamine. (2) *β-Hydroxylation.* A hydroxyl group on the β-carbon atom diminished potency: thus hydroxyphenethylamine was one-tenth as active as phenethylamine and phenylpropanolamine half as active as amphetamine. (3) *Stereoisomerism.* Optical activity here relates to the asymmetrical α-carbon atom. The dextro-form was more active than the laevo-form for excitatory amines. Thus, dexamphetamine was twice, and (+)-α-methyltryptamine four times as potent as the respective laevo-isomers. (4) *Variations in chain length.* Of the compounds tested with one, two and three carbon atoms between the ring structure and the terminal

nitrogen atom, *m*-methoxyphenethylamine (1.0 μmole/100 g) was the most active, eliciting electrocortical alerting and slight wing droop; at the same dose level, 3-(*m*-methoxyphenyl)propylamine and *m*-methoxybenzylamine were ineffective. Two carbon atoms between the ring structure and the terminal nitrogen were apparently necessary for optimal excitant activity.

Ring substitution. (1) *Alterations in the ring.* Compounds with various planar rings (cyclopentyl, phenyl or indolyl) and an aliphatic side-chain had excitant properties. Tetrahydronaphthylamine also had excitant properties. Potency corresponded with ring size; thus, on the electrocorticogram (+)-α-methyltryptamine was four times as potent as dexamphetamine and forty-two times as potent as (±)-cyclopentamine. (2) *Phenolic hydroxyl groups.* Hydroxylation in the 4-position as in hydroxyamphetamine rendered the substance inactive. (3) *Methoxy and methyl substituents.* 3-(*m*-Methoxyphenyl)-ethylamine and (+)-*m*-methoxy-α-methylphenethylamine had excitant activity but this was one-hundredth that of the corresponding molecule without the substituent on the ring.

Other behavioural effects

Postural changes

In addition to the effects on the electrocorticogram, the excitant amines cyclopentamine, tetrahydronaphthylamine, tuaminoheptane, α-methyltryptamine and amphetamine produced characteristic changes in posture and evoked twittering. These changes have been described in detail for α-methyltryptamine and for amphetamine (Dewhurst & Marley, 1965a). The threshold intravenous doses (in μmoles/100 g) were for (±)-tuaminoheptane, 5.0, and for (±)-cyclopentamine, 10.0, compared to 0.5 and 1.0 for α-methyltryptamine and amphetamine respectively. In general, the dose required to elicit these effects was two to three times larger than for threshold effects on electrocortical or electromyographic activity.

Twittering

Twittering was also evoked by these amines. The threshold doses for the effects on twittering lay between those for the effects on cheeping and electrocortical activity and those for postural changes. There was a delay of 10 min after injection before twittering developed. As (+)-methyltryptamine was the most potent of the amines eliciting twittering and the effect was prevented or abolished by methysergide and not by other specific antagonists, an action on central tryptamine receptors was apparently essential.

Tachyphylaxis and excitant amines

Little is known of tachyphylaxis to the central action of sympathomimetic amines. The effect of repeated doses of amphetamine on behaviour was studied first. Two birds were injected intraperitoneally every 5 hr, the one with saline and the other with dexamphetamine (3.0 μmoles/100 g), and observed continuously for 30 hr. Cheeping in the control bird waxed and waned in the 2 to 3 hr after each injection but was always considerable. In the test bird the first three doses of amphetamine increased cheeping and did not affect posture, and the fourth to sixth doses failed to evoke cheeping, suggesting tachyphylaxis. The bird was left for 10 hr without injection when amphetamine was again effective.

The effect of amphetamine on cheeping may be due to extraneous as well as to drug factors, whereas that on posture or the electrocorticogram depends only on the drug. To ensure that the declining effects of amphetamine were compatible with tachyphylaxis, a larger dose (6 μmoles/100 g, intraperitoneally) which would affect posture as well as cheeping was given every 5 hr to another chicken for 30 hr. The effects of a single dose were initially threefold. First, cheeping continued unaltered or was increased; after a lapse of 10 min, cheeping ceased as ataxia and postural changes developed (Fig. 1,*a*); finally, in the postataxic phase, cheeping returned. With repeated doses the pattern changed so that by the third dose (Fig. 1,*b*) the onset of ataxia and the postural changes with amphetamine was swifter and they were of longer duration as was postataxic cheeping. With further doses still, the effect of amphetamine dwindled with progressively delayed onset (Fig. 1,*d* to *f*) and waning ataxic and postural changes (Fig. 1,*c* to *f*). Postataxic cheeping was first increased in intensity (Fig. 1,*b*) then declined (Fig. 1,*c*,*d*) and finally did not occur (Fig. 1,*e*,*f*) although cheeping was elicited on handling the bird (Fig. 1,*f*). In contrast, but not shown, cheeping of the control bird increased with each injection of saline.

Tachyphylaxis to amphetamine (1 μmole/100 g) given intraperitoneally every 24 hr was not obtained, but was sometimes elicited if the amphetamine was injected intravenously. In such an experiment, the first three doses of amphetamine enhanced activity and cheeping with immediate electrocortical alerting. The fourth dose did not affect activity or cheeping; electrocortical alerting was delayed in onset for 18 min and was less intense when it did develop. Tachyphylaxis was also seen with cyclopentamine, tetrahydronaphythylamine, tuaminoheptane, 3-(*m*-methoxyphenyl)isopropylamine and α-6-dimethyltryptamine.

Cross-tachyphylaxis

Cross-tachyphylaxis developed between a central excitant drug with a phenyl ring and one with an indolyl ring. Thus (+)-3-(*m*-methoxyphenyl)isopropylamine (1 μmole/100 g) evoked sustained electrocortical alerting, cheeping and postural changes. In the same bird, 8 hr later, (+)-α-methyltryptamine (0.5 μmole/100 g), which would normally have had marked excitant actions, was ineffective; a further 1.0 μmole/100 g given 5 min afterwards produced minimal electrocortical alerting. However, intense electrocortical alerting and characteristic changes in cheeping and posture, but with delayed onset, were evoked by (+)-α-methyltryptamine (1 μmole/100 g) given 30 min later. Recovery was complete in 4 hr. Tachyphylaxis did not extend to an indolethylamine with a ring substituent, for (+)-6-methoxy-α-methyltryptamine (1 μmole/100 g) given to the same chicken produced sleep and electrocortical slow potentials and abolished cheeping. This was further evidence for the excitant and depressant amines exerting their effects through different receptors.

Pharmacological antagonism

Antagonists at tryptamine receptors

The excitant effects on cheeping and electrocortical activity of tryptamine or phenethylamine given in intravenous doses of 0.001 and 0.1 μmole/100 g were antagonized in two experiments by methysergide (0.01 μmole/100 g). In separate experiments, the electrocortical alerting, twittering and postural changes produced by intravenous doses of (±)-tuaminoheptane (10 μmoles/100 g), (±)-cyclopentamine (15 μmoles/100 g) and (±)-α-6-dimethyltryptamine (0.02 μmole/100 g) were antagonized by methysergide

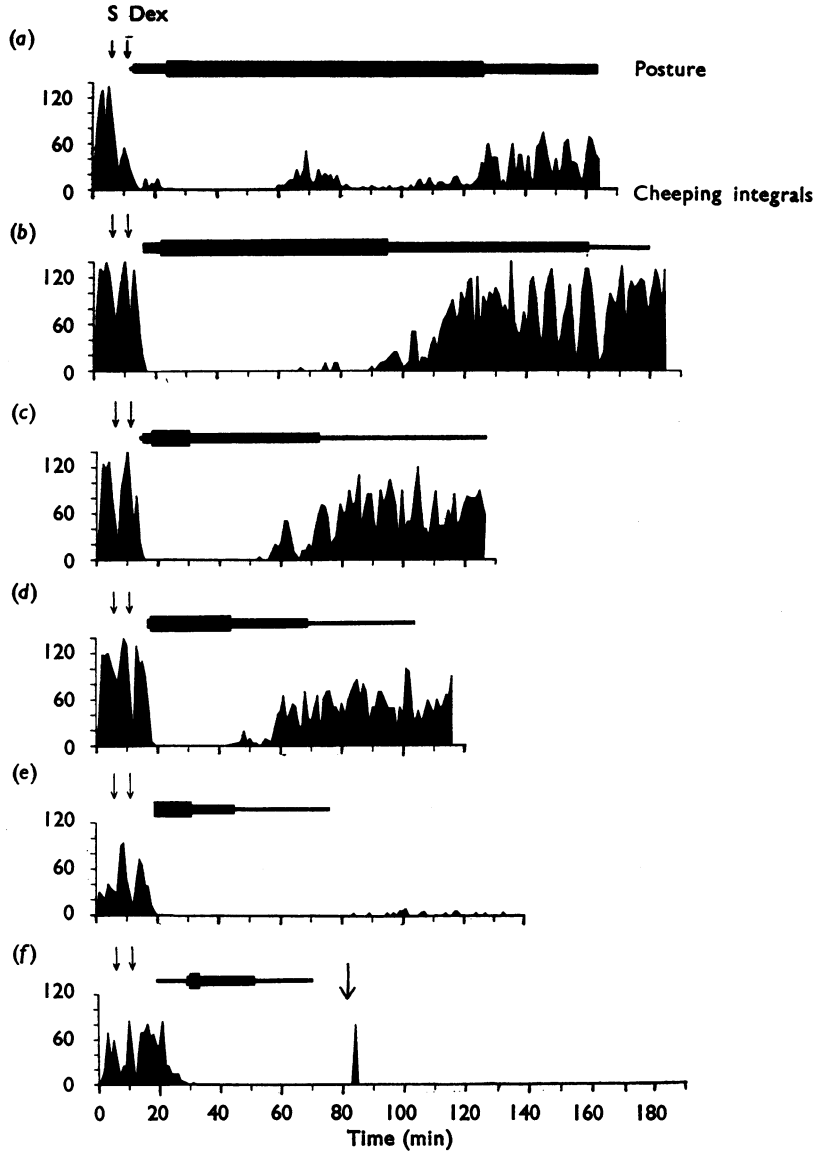

Fig. 1. Tachyphylaxis with repeated doses of dexamphetamine. Six (*a–f*) of seven consecutive doses of dexamphetamine (Dex, 6 μmoles/100 g, at second arrows) and of 0.2 ml. of saline (S, at first arrows) injected intraperitoneally every 5 hr for 30 hr in a 38-g chicken (second dose not shown). Effects on posture (upper bars) and cheeping (histograms below) are shown.

Postural changes are graded by the width of bar. Thin bar (Grade 1): slight postural change. Chicken mobile and capable of holding trunk above floor; wings held away from side. Medium bar (Grade 2): chicken still mobile but unable to raise belly from ground. Thick bar (Grade 3): marked postural change. Head and neck fixed with neck retraction. Chicken immobile with belly on ground. No bar: normal posture. Postural effects diminish in intensity and duration after the third dose (*b*) of dexamphetamine. With the first dose (*a*) the postural changes last about 140 min with Grade 3 changes for 100 min. With the seventh dose (*f*) the postural changes last only 55 min with Grade 3 effects for 2 min.

Abolition of cheeping: cheeping is in abeyance when postural changes are maximal. As shown for four of the first five doses of dexamphetamine (*a*, *b*, *c* and *d*) the progressively less intense and shorter duration of postural changes are associated with progressively shorter periods during which cheeping is abolished. The abolition of cheeping occurred more rapidly with the first four doses of dexamphetamine (*a*, *b* and *c*) and progressively more slowly with subsequent doses (compare *d*, *e* and *f* with *c*). Cheeping following postural changes: this is increased in intensity in *b* and *c* but absent in *e* and *f*. The absence of cheeping was not due to neuromuscular paresis as it could be elicited on handling the animal (*f*, at third arrow).

(0.001 μmole/100 g). Antagonism to (±)-tuaminoheptane lasted 128 min when it was surmounted by a further 10 μmoles/100 g of tuaminoheptane. Antagonism to (±)-cyclopentamine waned after 8 min and its excitant effects returned. A further intravenous dose of methysergide (0.004 μmole/100 g) produced antagonism for 25 min. The antagonism to (±)-α-6-dimethyltryptamine lasted 90 min, and the excitant effects then reappeared.

Methysergide (0.001 and 0.02 μmole/100 g) enhanced in three experiments the depressant effects of indole amines with ring substituents (6-hydroxytryptamine, 0.5 μmole/100 g, or 6-methoxy-α-methyltryptamine, 2 μmoles/100 g) and transiently antagonized the effect of 6-chloro-α-methyltryptamine (0.5 μmole/100 g).

Antagonists at α-receptors for catechol amines

Phenoxybenzamine given acutely showed little effect as an antagonist. Various methods of pretreatment were tried. With intravenous doses of 2 μmoles/100 g, coma was induced in young birds, but older birds (5 months) given 3 μmoles/100 g showed a moderate reversible hypotension, but no subsequent block of the pressor effects of 0.01 μmole/100 g of adrenaline. Previous treatment of the young chicken for 24 hr with 14 μmoles/100 g (intravenously in divided doses) antagonized the pressor response to adrenaline (0.01 μmole/100 g) but sleep followed in customary fashion. Isoprenaline and phenylephrine also produced drowsiness unaffected by previous phenoxybenzamine. Treatment for 4 days with ten intravenous doses of 0.75 μmole/100 g phenoxybenzamine had no effect on the sleep produced by 0.01 μmole/100 g adrenaline, or on the biphasic excitant-depressant response to 5-hydroxytryptamine (0.5 μmole/kg).

Hydergine had no effect on the responses to adrenaline or tryptamine. 6-Hydroxytryptamine (0.5 μmole/100 g) preceded 30 min earlier by mebanazine (10 μmoles/100 g) which intensified its depressant action was also unaffected by Hydergine (0.25 μmole/100 g).

Similarly, dihydroergotamine (0.25 μmole/100 g) had no effect on the central depressant action of adrenaline (0.01 μmole/100 g) although the pressor effects were blocked.

Antagonists at β-receptors for catechol amines

Pronethalol (0.5 μmole/100 g, intravenously) did not antagonize the drowsiness induced by isoprenaline (0.1 and 0.2 μmole/100 g, intraperitoneally, 20 min later), or by phenylephrine (2 μmoles/100 g, intravenously, 20 min later). The soporific effect of (−)-adrenaline (0.01 μmole/100 g, intravenously) was slightly reduced after 5 μmoles/100 g pronethalol but at this dosage pronethalol had excitant effects and physiological rather than specific antagonism was probably responsible.

Propranolol (0.01 and 0.1 μmole/100 g) had no effect on the depressant response to noradrenaline (0.01 μmole/100 g). Dichloroisoprenaline (1 μmole/100 g), unlike pronethalol, had depressant agonistic properties but subsequent doses of (−)-adrenaline (0.01 μmole/100 g) had unimpaired responses.

Potentiation

Central depressant amines

The effects of adrenaline were potentiated by cocaine whereas those of tyramine were potentiated by monoamine oxidase inhibition.

Thus, electrocortical and behavioural sleep lasting for 5 min was evoked by (−)-adrenaline (0.005 μmole/100 g). The duration of sleep produced by this dose was doubled after cocaine (1 μmole/100 g) which by itself did not affect behaviour and electrocortical activity, whereas the electrocortical alerting evoked by phenethylamine (1 μmole/100 g) or tryptamine (0.1 μmole/100 g) was unaltered. The potentiating effect of cocaine on the central depressant effects of (−)-adrenaline were confirmed in a second experiment.

A dose of paratyramine (2 μmoles/100 g) usually induced behavioural and electrocortical sleep lasting 2 min. After the amine oxidase inhibitor mebanazine (10 μmoles/100 g, 60 min previously), which by itself was ineffective, the sleep induced by paratyramine lasted 30 min (two experiments).

Intermediate group of amines

Both the excitant and depressant phases elicited by 5- or by 6-hydroxytryptamine were prolonged by amine oxidase inhibition (five experiments). Thus 60 min after mebanazine (10 μmoles/100 g), electrocortical alerting evoked by 6-hydroxytryptamine (0.5 μmole/100 g) lasted 3 min (normal duration, 1 min) and this was followed by sleep for 30 min (normal duration, 5 min) accompanied by large-amplitude 2- to 4-cycles/sec electrocortical potentials.

Central excitant amines

The electrocortical alerting evoked either by phenethylamine or by tryptamine (0.5 μmole/100 g) usually lasted 5 to 10 min. At 60 min after mebanazine (10 μmoles/100 g), 0.5 μmole/100 g of phenethylamine or tryptamine evoked twittering, marked postural changes and electrocortical alerting lasting 30 min. The results, which were similar to those produced by the α-methyl derivatives of phenethylamine or tryptamine in the absence of amine oxidase inhibition, were confirmed in four chickens.

Synergism

Pronethalol (10 μmoles/100 g) potentiated the effect of amphetamine on cheeping. Tests were made in two isolated chickens which cheeped little, if at all, after dexamphetamine (3 μmoles/100 g). As shown in Fig. 2,*a* cheeping was much more marked with pronethalol followed by the dose of amphetamine than with the same dose of amphetamine given alone (Fig. 2,*b*). The small effect with the second dose of amphetamine was not due to tachyphylaxis since, in another chicken, amphetamine given first (Fig. 2,*c*) had a much smaller effect on cheeping than amphetamine given on the following day after pronethalol (Fig. 2,*d*). The effects of (+)-α-methyltryptamine were also enhanced after pronethalol.

Amino acid precursors of noradrenaline and tryptamine

The precursor of noradrenaline, dopa, in intravenous doses of 5 or 10 μmoles/100 g, alerted a chicken for 15 to 20 min; a dose of 1 μmole/100 g was ineffective. Similar results were obtained with these doses on the following day and the effects were enhanced by the prior injection of the amine oxidase inhibitor pargyline (5 μmoles/100 g). Dopa (10 μmoles/100 g) elicited electrocortical alerting for 10 min in another bird tested.

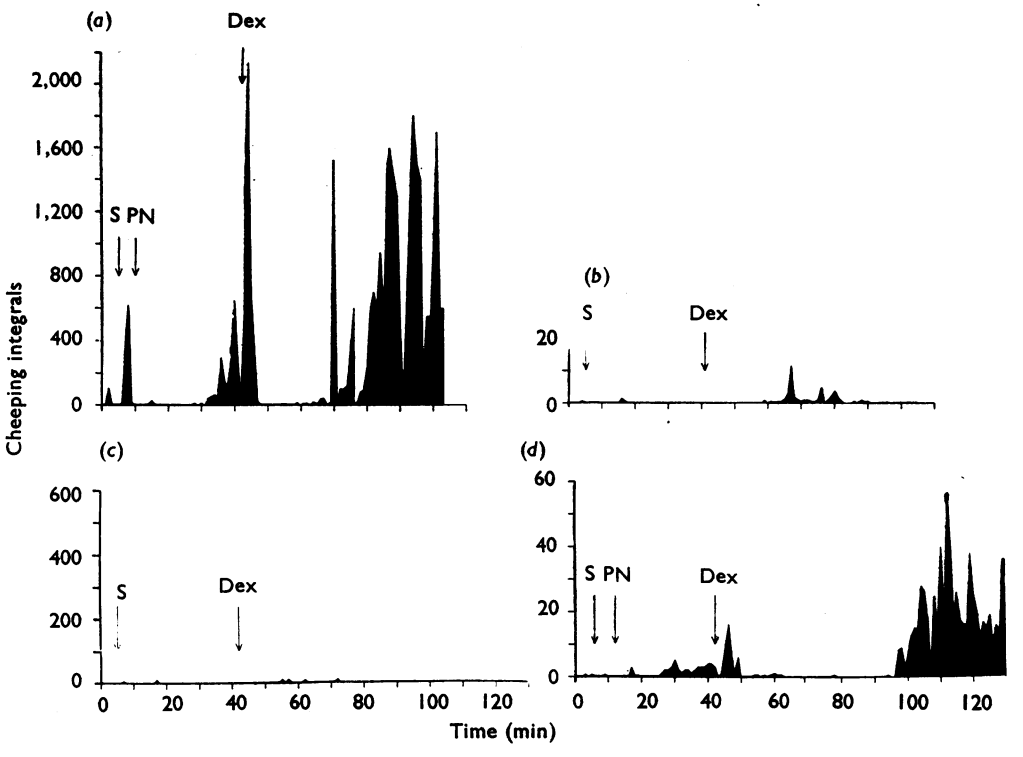

Fig. 2. Histograms for cheeping showing synergism between dexamphetamine and (±)-pronethalol. (*a*) and (*b*), first chicken. Much more cheeping in (*a*) following pronethalol (PN, 10 μmoles/100 g) and dexamphetamine (Dex, 3 μmoles/100 g) than in (*b*) 24 hr later when dexamphetamine was given after saline (S, 0.2 ml.). (*c*) and (*d*) second chicken. Much less cheeping with dexamphetamine (3 μmoles/100 g) given after saline (0.2 ml.) in (*c*) than in (*d*) 24 hr later when given after pronethalol (10 μmoles/100 g). All injections were intravenous.

The precursor of tryptamine and of 5-hydroxytryptamine, L-tryptophan, in doses of 2 and 5 μmoles/100 g, produced drowsiness for 3 min followed by electrocortical alerting and increased cheeping for 10 min. After pargyline (5 μmoles/100 g) a dose of L-tryptophan, which had previously been ineffective (1 μmole/100 g), produced drowsiness with large-amplitude slow electrocortical potentials lasting 1 min followed by electrocortical arousal and augmented cheeping lasting 20 min.

Adult fowl

Some sympathomimetic amines have been tested in the adult fowl (Key & Marley, 1962) but the indoleamines were not included. The study showed that the effects of some amines consistently differed between young and adult chickens. For this reason additional experiments were made on the adult fowl with tryptamine derivatives having depressant or excitant properties.

In a drowsy adult bird (±)-6-methoxy-α-methyltryptamine (10 μmoles/kg) elicited behavioural and electrocortical alerting and tachypnoea for about 2 min. Subsequently the

bird squatted with closed eyes and the electrocorticogram displayed large-amplitude slow waves for 80 min; sensory stimuli evoked brief arousal. The brief excitant followed by long-lasting depressant effects were similar to those observed in 1- to 28-day-old chickens. At this point (+)-α-methyltryptamine (5 μmoles/kg) was injected. Intense and immediate

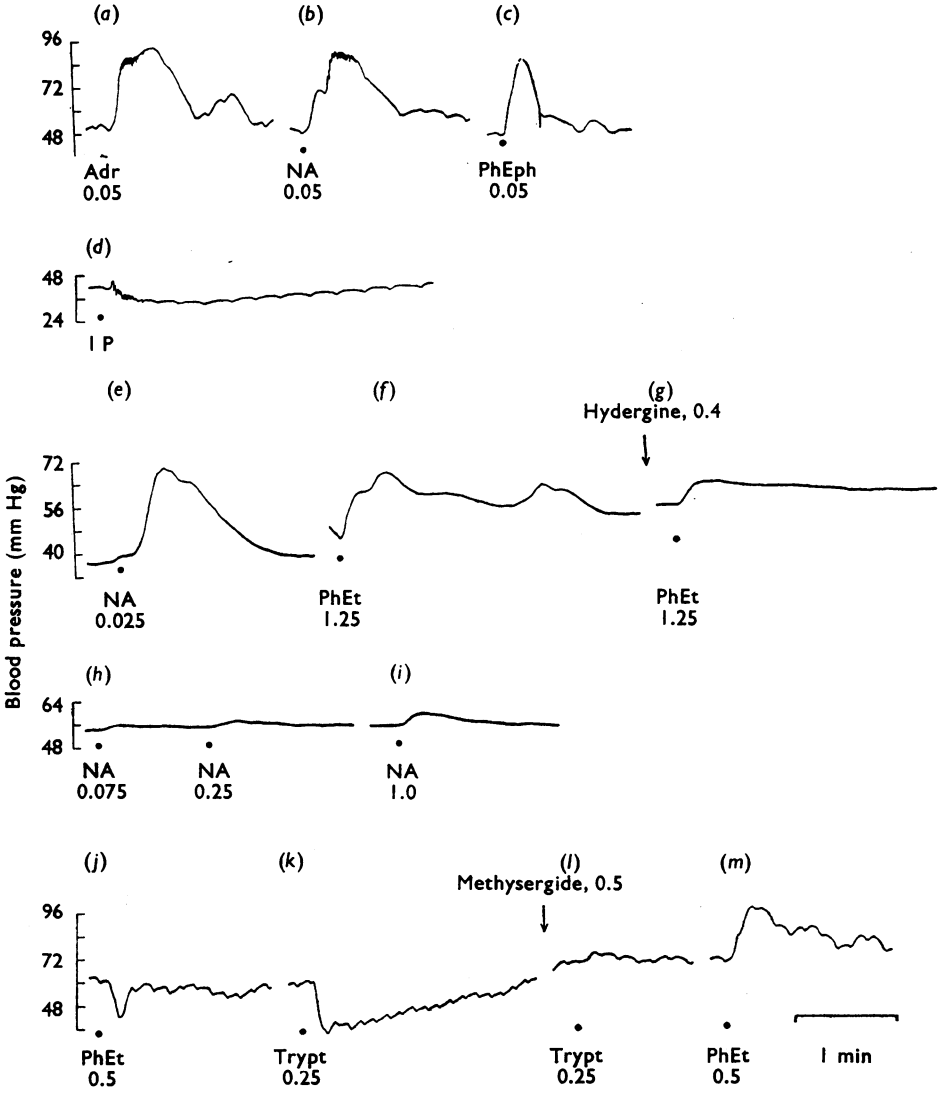

Fig. 3. Blood pressure response to amines. (*a*) to (*d*), spinal chicken, 1.1 kg. Pressor activity declines in the order (−)-adrenaline (Adr), (−)-noradrenaline (NA) and (−)-phenylephrine (PhEph); (−)-isoprenaline (IP) is feebly depressor. (*e*) to (*i*), spinal chicken, 2.0 kg. Pressor activity of (−)-noradrenaline and phenethylamine (PhEt) is antagonized by Hydergine; antagonism to phenethylamine (*g*) is more readily surmounted than that to noradrenaline (*h*, *i*). (*j*) to (*m*), spinal chicken, 1.0 kg. Depressor activity of tryptamine (Trypt) and phenethylamine; the effect of tryptamine is antagonized by methysergide whereas the pressor action of phenethylamine is uncovered. All doses are given as μmole/kg and were injected intravenously.

electrocortical alerting ensued lasting 100 min. The wings were extended, the head was retracted, the beak was agape and the trunk rested on the floor but with the tail elevated, a posture also elicited in the young chicken by (+)-α-methyltryptamine. The results were confirmed in two adult fowls. In another adult chicken, tryptamine (5 μmoles/kg) injected 30 min after an amine oxidase inhibitor, mebanazine (100 μmoles/kg), elicited similar electrocortical and postural effects to α-methyltryptamine.

Effects of amines on blood pressure

The pressor activity of the catechol amines declined in the order (−)-adrenaline, (−)-noradrenaline and (−)-phenylephrine (Fig. 3,*a* to *d*); adrenaline and noradrenaline being almost equiactive; isoprenaline was feebly depressor.

The excitant amines were much less uniform in their effects than the catechol amines. The changes in blood pressure produced by phenethylamine and tryptamine are illustrated in Fig. 3. Tryptamine had striking depressor activity (Fig. 3,*k*) although this was often preceded or followed by a small blood pressure rise; occasionally small doses (0.25 μmole/kg) were entirely pressor. The hypotensive action was not due to a von Bezold-like reflex as it persisted after the vagi had been cut and the brain destroyed. Phenethylamine had less consistent effects than tryptamine; sometimes it was pressor (five out of ten experiments; Fig. 3,*f*), sometimes biphasic (three experiments) and sometimes depressor (two experiments; Fig. 3,*j*). The hypotensive effect of intravenous tryptamine or phenethylamine was reduced, or converted to a rise in blood pressure, when the amine was injected so that it reached the systemic before the pulmonary circulation. This accords with the suggestion by Eble (1963) that tryptamine produces pulmonary vasoconstriction with reduced transfer of blood from the right to the left side of the heart leading to a fall in systemic blood pressure.

The depressor action of tryptamine was rapidly reduced or abolished by the tryptamine antagonist methysergide (Fig. 3,*k* and *l*). Larger doses of methysergide were required than those sufficient for central antagonism, emphasizing the lack of correlation between the blood pressure and central effects. Antagonism was prolonged and surmountable with difficulty. The pressor action of phenethylamine was at most slightly diminished by methysergide in six tests; in one experiment there was considerable antagonism. The α-receptor antagonist Hydergine (0.3 to 0.6 μmole/kg) substantially reduced the pressor activity of phenethylamine and noradrenaline (Fig. 3,*e* to *i*). Antagonism to phenethylamine was less effective, more easily surmountable and more poorly sustained compared to antagonism of noradrenaline (Fig. 3,*h* and *i*). The blood pressure effects of noradrenaline, phenethylamine or tryptamine were not antagonized by chlorpheniramine (2 μmoles/kg), hyoscine (2 μmoles/kg) or pronethalol (25 μmoles/kg) even when the antagonist was given in a 100-times the dose of the agonist. This dose of pronethalol antagonized the effects of isoprenaline.

The fall of blood pressure following phenethylamine could be converted to a pressor action by methysergide (compare Fig. 3,*j* and *m*) suggesting that the depressor effect was due to an action on tryptamine receptors. The effect of tryptamine and α-methylphenethylamine on the blood pressure progressively declined when these amines were injected alternately every 10 min, also suggesting that they acted on similar receptors. After treatment

with the amine oxidase inhibitor, (±)-tranylcypromine (0.5 or 1 μmole/kg), tryptamine produced a rise instead of a fall of blood pressure but the pressor activity of ensuing doses of phenethylamine declined (three out of four experiments) indicating cross-tachyphylaxis. After reserpine (10 μmoles/kg, intraperitoneally, 24 hr previously), the chicken was extremely sensitive to tryptamine, an intravenous dose of 0.02 μmole/kg often producing fatal falls of blood pressure. Reserpine also uncovered the probable action of phenethylamine on tryptamine receptors for its effects were now depressor (all of four experiments).

Relatively large doses of cocaine (25 to 50 μmoles/kg) were required to antagonize the effect of phenethylamine, although the pressor action of the catechol amines was significantly enhanced by cocaine (2.5 μmoles/kg).

A summary of the findings for the effects of the amines on the central nervous system and on the blood pressure is given in Table 2.

TABLE 2

CENTRALLY MEDIATED ACTIONS OF EXCITANT AND DEPRESSANT AMINES COMPARED AND RELATED TO MOLECULAR STRUCTURE

ECoG and EMG = electrocortical and electromyographic activity. Threshold doses are intravenous

	Depressants	Excitants
Response		
Behaviour	Drowsy or sleeping	Active, exploratory, aggressive
Vocal activity	Diminished or absent	Increased
Locomotor system and posture	Diminished activity, normal posture	Increased activity but postural and ataxic changes with high doses
Tremor and ataxia	Antagonizes centrally induced tremor	Produces tremor
ECoG amplitude	Increased	Diminished
ECoG frequency	Diminished	Increased
EMG amplitude	Diminished	Increased
Optimal structure and activity		
Most potent, and threshold dose	(−)-Adrenaline, 0·001 μmole/100 g	Tryptamine, 0·001 μmole/100 g Phenethylamine, 0·01 μmole/100 g
N-Substituent	$-NH.CH_3$ optimal	$-NH_2$ optimal
α-Substituent	Increases duration of action, diminishes potency	Increases duration of action Diminishes potency
β-Substituent	One –OH optimal	Not hydroxylated
Ring	3,4-Dihydroxyphenyl optimal (? indolyl)	Lipid soluble, aliphatic, alicyclic, aromatic or heterocyclic not hydroxylated,
Stereoisomerism	Laevo-form (referential to β-carbon atom) most potent	Dextro-form (referential to α-carbon) most active
Tachyphylaxis	None	Occurs
Cross-tachyphylaxis within same group	None	Occurs
Cross-tachyphylaxis with opposing group	None	None
Synergism	Within same group	Within same group and with pronethalol
Potentiation	By cocaine, or by monoamine oxidase inhibition	By monoamine oxidase inhibition
Physiological antagonist	Excitants	Depressants
Specific antagonist	? α-Blockers	Methysergide

Chromatography

Mean R_F values are listed in Table 1. The partition coefficient was calculated from the expression $\alpha_m = (A_s/A_m)\ [R_F/(1-R_F)]$ which was derived from the expressions given by Bush (1961) and Martin & Synge (1941), where A_m and A_s are the cross-sectional areas of the mobile (m) and stationary (s) phases. For the present experiments, A_s/A_m was a constant = 1.95; α_m is the partition coefficient of the amine expressed as concentration in (m) divided by concentration in (s).

The results show that separation of the amines into three groups by their biological effects also generally held for partition coefficients. A partition coefficient of 0.7 or more was obtained for the central excitant amines, and of 0.5 or less for the central depressant amines. Values between the two extremes were found for the intermediate group of amines. Three substances appear anomalous. 5-Hydroxytryptamine and 6-hydroxytryptamine had partition coefficients characteristic of depressants although possessing some biologically excitant properties. However, after potentiation by amine oxidase inhibitors, depressant activity was dominant. 6-Chloro-α-methyltryptamine conversely had marked excitant effects and its depressant activity was less important.

DISCUSSION

Barger & Dale (1910) defined sympathomimetic amines as a group of substances with qualitatively similar actions to sympathetic stimulation but with quantitative differences in activity. This view has been modified and it is now held that the catechol amines act directly on specific receptors whereas other amines, such as amphetamine, act indirectly by causing noradrenaline release (Burn, 1960). In certain circumstances, however, an action of the amphetamine-like amines on peripheral tryptamine receptors has been demonstrated (Vane, 1960). As the catechol- and amphetamine-like amines produce similar central effects in adult animals it has been assumed that they acted in an identical manner at the same site in the brain, probably in a similar way to their action at peripheral receptors. From experiments in the young chicken (Key & Marley, 1962; Dewhurst & Marley, 1964) it was clear that in this species the catechol- and the amphetamine-like amines had different modes of action. This work has now been extended, especially with respect to the importance of central tryptamine receptors.

The central depressant amines possessed an aliphatic side-chain attached to a substituted phenyl or indolyl ring. The side-chain was essential, for compounds that were similar but lacked the side-chain such as pyrogallol (Key & Marley, 1962) and catechol had inconsistent effects. A basic terminal only to the side-chain was important, for dopa with an acidic as well as a basic terminal had excitant activity. It has been assumed that the central actions of dopa are through its conversion to dopamine and to noradrenaline. This is unlikely in the chicken, for dopa was excitant whereas dopamine and noradrenaline were central depressants. However, in rodents, increased motor activity following the injection of dopa has been related to an increased concentration of dopamine in the brain (Everett, 1961; Dagirmanjian, Laverty, Mantegazzini, Sharman & Vogt, 1963). That the excitant effects of dopa were enhanced by treating the animal with a monoamine oxidase inhibitor also suggested that the effects were due to the conversion of dopa to dopamine, since amine oxidase is important in the inactivation of dopamine (Blaschko, 1956). Yet dopa retained central excitant activity in mice treated with α-methyldopa (Smith, 1963) which prevents the

decarboxylation of dopa to dopamine. This, and the findings in the chicken, suggest that dopa acts directly on neurones or peripheral vascular receptors and is not simply an inert precursor of dopamine, noradrenaline or adrenaline. Confirmation of this view comes from experiments in which dopa was applied iontophoretically and increased cortical neuronal firing in the cat, whereas dopamine, noradrenaline and adrenaline depressed discharge (Krnjević & Phillis, 1963).

The same alterations in chemical structure which modify the potency of the amines on peripheral receptors in mammals determined activity in the central nervous system. For example, central depressant potency declined in molecules with alkyl substituents on the terminal nitrogen or on the α-carbon atom, or in the absence of the β-hydroxyl group. Optical activity also determined potency. Amines with methoxy- or chloro-substituents on the phenyl ring were central depressants. Crucial compounds to test, had they been available, would have been indolealkylamines with two or more hydroxyl groups on the radical, for these might well be as potent or more so than the catechol amines. Indolealkylamines with single electronegative substituents in these positions had brief excitant followed by longer-lasting depressant activity. The production of behavioural and electrocortical sleep by amines with substituted phenyl or indolyl ring structures suggested that they acted through similar mechanisms. Receptors common to adrenaline and 5-hydroxytryptamine occur in a number of systems (Innes, 1962).

The decreasing central depressant activity of the series (±)-6-chloro- and (±)-6-methoxy-α-methyltryptamine and the excitant properties of (±)-α-6-dimethyltryptamine suggested that the electronegativity of the substituent determined depressant activity. Electronegativity declines in the order chloro-, hydroxy-, methoxy-group, whereas the methyl group is electropositive (Heys, 1960). The central depressant amines should perhaps be considered as having a positive charge at one end and a negative charge at the other end of the molecule.

If receptors are specified by order of potency of agonist as recommended by Ahlquist (1948) then depressant amines, with an order (−)-adrenaline > (−)-noradrenaline > (−)-isoprenaline, could be presumed to act on central α-receptors. A similar order of potency also applies for their pressor activity in the spinal chicken and these blood pressure effects were blocked by Hydergine, an α-receptor antagonist.

Antagonists at α-receptors such as phenoxybenzamine were ineffective against the central depressant effects of most catechol amines tested, although, as previously reported (Dewhurst & Marley, 1965a), phenoxybenzamine had antagonist actions against α-methylnoradrenaline. Harvey & Nickerson (1951) observed that surprisingly large doses of dibenamine were required to abolish the pressor actions of adrenaline in the fowl. It is becoming clear that large doses of phenoxybenzamine given over a number of days are required for effective antagonism against the central depressant effects of the catechol amines, for, given these conditions, the depressant effects of α-methylnoradrenaline on pecking under operant conditions are antagonized (Marley & Morse, unpublished) as are its hypothermic actions (Allen & Marley, 1965).

In the young chicken the peripheral and central actions of the catechol amines could be dissociated since they elicited sleep even when their pressor action had been abolished by pharmacological antagonists. In adult animals it is difficult to dissociate the central from

the peripheral actions of these amines injected intravenously. Thus phentolamine, which blocks the peripheral action of adrenaline, also suppresses the electrocortical alerting which would otherwise occur (Capon, 1960). This implied either that the central and peripheral receptors were similar in characteristics or that the central action of adrenaline was secondary to its peripheral activity. Some workers believe that catechol amines have a direct central excitant action (Bonvallet, Dell & Hiebel, 1954; Dell, 1960; Cordeau, Moreau, Beaulnes & Laurin, 1963). Others believe that the amines have an indirect excitant action by initiating afferent impulses elsewhere in the body which affect the midline structures in the brain-stem (Mantegazzini, Poeck & Santibañez, 1959; Feldberg, 1960; Baust, Niemczyk & Vieth, 1963). The evidence for either view has been summarized by Bradley (1960). Our results suggest that if the catechol amines penetrate to the brain (after intravenous injection into young chickens or by intraventricular injection into adult animals) they produce sleep either by depressing neuronal activity or by activating sleep mechanisms, but that when alerting occurs (after intravenous injection in adult animals) it is primarily secondary to their peripheral action. Iontophoretic application of the amines to neurones should provide critical evidence, but it is difficult to relate the effects to behaviour or to changes in electrocortical activity. There is also a considerable difference between doses given directly on to, or into, neurones and those given intravenously to intact animals. The concentration of tryptamine and phenethylamine derivatives applied iontophoretically and calculated to be achieved at cortical neurones to depress discharge was 10^{-4} M (Krnjević & Phillis, 1963). This was much larger than in experiments with the chicken in which effective doses for excitant or depressant amines ranged from 10^{-9} to 10^{-6} M/100 g body weight.

The central excitant amines possessed an aliphatic side-chain attached to one of a number of lipophilic radicals which were aromatic (phenyl, indolyl), straight-chain aliphatic (tuaminoheptane), alicyclic (cyclopentyl) or mixed (tetrahydronaphthyl). With few exceptions the radical was unsubstituted; substituents on the ring or on the α- or β-carbon atoms diminished potency. That tuaminoheptane had central excitant activity and that potency diminished with large substituents on the aliphatic portion of the molecule as in methyl phenidate, phenmetrazine and pipadrol (Key & Marley, 1962), suggested that central excitant as well as depressant compounds orientated to the receptor through their cationic terminal nitrogen atom presumably by electrostatic forces. A methyl or larger substituent on the α-carbon atom diminished potency as with the central depressant amines, presumably due to steric hindrance or to redistribution of ion charge.

In compounds with constant length of side-chain, potency followed ring size. Cyclopentamine, amphetamine and α-methyltryptamine showed increasing activity. The ring structure in each case was planar, so van der Waal's forces probably contributed to attachment at the receptor. As α-methyltryptamine was more active than amphetamine, weak ionic bonding by the partially charged ring nitrogen may also occur. As in the experiments of Barger & Dale (1910) with smooth muscle, optimal activity was present when two carbon atoms intervened between the ring and the terminal nitrogen.

In the chicken, tryptamine was the most potent of the excitant amines. In addition it shared a number of physiological and behavioural responses possessed by a variety of excitant amines. That antagonism of all amines in the excitant group could be accomplished by methysergide strongly implied that these excitant effects were mediated by similar

receptors and supported the suggestion by Vane (1960) and Gelder & Vane (1962) that amphetamine acts on central tryptamine receptors.

Whereas the central effects of phenethylamine and tryptamine were similar their actions on blood pressure differed. Tryptamine usually produced a fall in blood pressure antagonized by methysergide. The fall in blood pressure was probably due to pulmonary vasoconstriction (Eble, 1963). Histamine release has also been suspected (Buñag & Walaszek, 1962) but there was no evidence for this in man (Dewhurst, 1965). The sudden deaths of healthy chickens after injection of α-methyltryptamine (Dewhurst & Marley, 1965a) and of tryptamine were presumably a consequence of this fall in blood pressure. Phenethylamine normally had pressor or biphasic actions antagonized by Hydergine, although there appeared to be a subsidiary action of phenethylamine on tryptamine receptors. Sudden deaths have also occurred on injection of amphetamine.

These observations exclude any causative relation between the blood pressure effects and the primary central actions of these amines as defined. Thus, adrenaline and phenethylamine both raised the blood pressure but one was a central depressant and the other a central excitant. On the other hand, isoprenaline and tryptamine both lowered the blood pressure yet one was a central depressant and the other had central excitant activity. This is not to say that cardiovascular changes do not have cerebral effects but that such effects are secondary to peripheral actions and must be excluded to establish a primary cerebral action.

The structure and classification of the molecules best fitting central receptors in the young chicken were very similar to those described by Vane (1960) for the smooth muscle of the rat stomach. There was no need in the case of either the chicken or the rat stomach to differentiate between directly and indirectly acting sympathomimetic amines for the results were compatible with a direct action of the amines on receptors. The difference in the effects of the central excitants and the depressants in the chicken could be explained entirely by the differences in their structure and their electronic properties.

The chromatographic findings give an indication of the importance of physical properties. The partition coefficient between fat (olive oil) and water has long been considered of vital importance in connection with substances acting on the central nervous system (Meyer, 1899; Overton, 1901). However, the use of olive oil has been criticized in the light of subsequent knowledge that cell membrane lipid constituents are comprised chiefly of aliphatic alcohols and phospholipids (Albert, 1960). Hence the use of oleyl alcohol as an unusual chromatographic solvent and the derivation of the partition coefficients between oleyl alcohol and the aqueous buffer. It was interesting and surprising that the compounds could be divided so well into excitants and depressants simply from their partition coefficients. In general the findings justify the assertion that physical properties of the amines were important determinants of the qualitative effect on the central nervous system, whereas potency within a particular category appeared to depend more closely on the chemical structure.

Our results allowed certain deductions about the combination of the amines with their receptors. The need for an aliphatic portion of the molecule in both excitant and depressant amines suggested there would be ion-pair formation between the positively charged nitrogen atom of the cationic head and negatively charged receptor sites in the brain. Belleau (1960)

proposed a similar mechanism for the interaction of phenethylamines with smooth muscle. There would also be secondary attachment to the rest of the receptor through the planar radical. In addition, for central depressant activity, electronegative substituents were required at least in the 3 and 4 position on the phenyl radical or the 5, 6 or 7 positions on the indolyl radical, suggesting that these were at least weakly ionized and therefore capable of ionic bonding to the receptor or determining solubility. As depressant activity of the catechol amines was increased further by the β-hydroxyl group, the latter appears to be involved in hydrogen bonding to the receptor. An interpretation of our experimental findings is given in Table 3.

TABLE 3

PROPOSED MECHANISMS OF ACTION OF CENTRAL DEPRESSANT AND EXCITANT AMINES

	Depressants	Excitants
Molecular mechanism		
Bonding to receptor	Ionic bonding through cationic head, $-N^+H.CH_3$ Van der Waal's bonding through the nucleus; hydrogen bonding of the β-hydroxyl group	Ionic bonding through cationic head, $-NH_3$; van der Waal's bonding through the nucleus
Essentials for activity	Ethanolamine with β-substituent possessing electronegative groups	Ethylamine with β-substituent, aliphatic or aromatic and possessing no or electropositive groups
Partition coefficient	0·5 or less	0·7 or more
Cellular action	Direct action on neurones, ? α-receptor	Direct action on neurones; tryptamine receptor
Alerting mechanism	With large doses alerting occurs probably due to the increase in blood pressure associated with activation of peripheral vascular α-receptors	Characteristic response due to direct action on central receptors; variable effects on blood pressure

Attempts to relate the central actions of drugs to their chemical structure have not generally been very fruitful. In the young chicken it has been possible to do this. As these findings were similar to those obtained with certain types of mammalian smooth muscle the results indicate a general biological validity.

SUMMARY

1. The effects of sympathomimetic amines, indoleamines and other amines, and their amino acid precursors, were tested in the chicken on electrocortical and electromyographic activity, cheeping, movement and posture. Responses were recorded simultaneously and continuously under controlled conditions.

2. In the 1- to 28-day-old chicken, the amines fell into three groups: (*a*) central depressant amines which produced physiological sleep with large-amplitude 1- to 4-cycle/sec electrocortical activity and diminished cheeping, movement and electromyographic activity; (*b*) central excitant amines which produced alert behaviour with low-amplitude 15- to 30-cycles/sec electrocortical activity and, in the case of amines with long-lasting effects, increased cheeping, twittering, characteristic postural changes and enhanced electromyographic potentials; and (*c*) a group intermediate structurally between the central

depressant and excitant amines with either mixed depressant and excitant actions, equivocal or sequential biphasic actions.

3. The effects of the three groups of amines were related to their chemical structure.

4. Central depressant effects were given by amines with an aliphatic side-chain attached to a phenyl or indolyl radical with hydroxy-, methoxy- or chloro-substituents. Adrenaline was the most potent of the series. The laevo- were more potent than the dextro-isomers of those tested, optical activity relating to the β-carbon atom. The depressant effects of the catechol amines were enhanced by cocaine. The order of potency of the central depressant amines suggested an action on central α-receptors. Pharmacological antagonists at α-receptors were relatively ineffective against the catechol amines. The central depressant amines were physiological antagonists to the excitant amines.

5. Central excitant amines were either aliphatic, as exemplified by tuaminoheptane, or consisted of an aliphatic component attached to an unsubstituted cyclopentyl, indolyl or phenyl ring. A minority of central excitants had substituents on the ring structure. Tetrahydronaphthylamine also had central excitant activity. Tryptamine was the most potent of the series. The dextro- were more potent than the laevo-isomers of those tested, optical activity relating to the α-carbon atom. In a homologous series, optimal excitant activity was obtained in molecules with two carbon atoms between the terminal amino group and the radical. The actions of the excitant amines were antagonized by the specific tryptamine antagonist, methysergide. The central excitant amines were physiological antagonists to the depressant amines.

6. The intermediate group consisted of amines with initial central depressant succeeded by central excitant activity (oxedrine, 5- and 6-hydroxytryptamine), of amines with central depressant activity in low dose and excitant actions with higher dose (6-chloro- and 6-methoxy-α-methyltryptamine, metaraminol and ($\pm$)-α-ethylnoradrenaline), and of amines that were apparently without activity.

7. Substituents on the α-carbon atom or those larger than a methyl group on the terminal amino-group diminished potency of both central excitant and depressant amines. The presence of a hydroxyl group on the β-carbon atom diminished potency of the excitant amines and increased depressant potency of the catechol amines.

8. Tachyphylaxis developed to the repeated injection of the central excitant amines, and there was cross-tachyphylaxis between the various molecules of this group. There was no cross-tachyphylaxis between the central excitant and depressant amines.

9. The amino acid precursors of noradrenaline and tryptamine, dopa and L-tryptophan had central excitant actions.

10. The catechol amines had pressor activity, adrenaline being the most potent. Their effect was antagonized by Hydergine which blocks α-receptors. The phenethylamine-like compounds had pressor, mixed depressor and pressor, or least frequently entirely depressor actions. The pressor action was antagonized by Hydergine and the depressor effect by the tryptamine antagonist, methysergide. Tryptamine had depressor activity abolished by methysergide. Cross-tachyphylaxis developed between the effects of the tryptamine and the phenethylamine-like amines on the blood pressure.

11. There was no apparent relation between the effects of the amines on the blood pressure and primary effects on the central nervous system.

12. The partition coefficients between oleyl alcohol and aqueous phase were 0.7 or higher for the central excitants and 0.5 or lower for central depressants. Values between the two extremes were found for the intermediate group of amines.

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