EFFECT OF CATECHOLAMINE PRECURSORS AND PHENYLETHYLAMINES ON SPONTANEOUS ACTIVITY AND ON THE RESPONSE TO METHOHEXITAL ANAESTHESIA*

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Science is built up by successive solutions given to questions of increasing subtlety, approaching nearer and nearer towards the very essence of phenomena

LOUIS PASTEUR, *Etudes sur la Bière*

SINCE von Euler established the functions of epinephrine and norepinephrine as adrenergic transmitters and with the finding of another tissue catecholamine—dopamine—which is a precursor of the first two, a great deal of interest has developed surrounding the pressor and psychic effects of these substances. It is now quite clear that epinephrine acts primarily as a hormone in the body, while norepinephrine, which is an intermediate of epinephrine formation, acts as a hormone also but is a nervous transmitter as well and is perhaps the most important substance in the body with respect to control of the cardiovascular system. The function of dopamine, aside from its role as an intermediate of norepinephrine formation, is not yet clear, although one may suspect that it has a physiological function of its own.¹

In the past decade, the endogenous catecholamines have been identified as the main substances in the body which can prevent or counteract the deleterious effects of some anaesthetics on the cardiovascular system, while they have been known to *cause* serious cardiac disturbances with other anaesthetics.^{2,3} During this period, work has also been directed towards determining whether epinephrine and/or some of its precursors play a role in the aetiology of psychomotor disturbances, but the information in this field is still confusing.⁴⁻⁶

Variations in the concentration of circulating catecholamines and changes produced in the arterial blood pressure by these also appear to influence the activity of the facilitatory and inhibitory portions of the reticular formation of the brain stem, which has a direct influence on wakefulness.^{7,8} These influences have been studied specifically by recording the electrical activity of the spinal motor neuron on preparations with brain stem sections at different levels.⁹ These experiments demonstrated that after premammillary transection of the brain the intravenous injection of epinephrine always produces a considerable facilitation of the monosynaptic reflex, of the spontaneous activity of the motor neuron, and less regularly, of the polysynaptic reflex. This facilitation no longer occurs after retromammillary transection of the brain and is abolished also by anaesthetic agents. After retropontine transection, the same injection of epinephrine produces a rapid and profound inhibition of the monosynaptic and polysynaptic reflexes.

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and of the spontaneous activity of the motor neuron. This inhibitory effect is replaced by facilitation when the roots of the IXth and Xth cranial nerves are sectioned intracranially to eliminate the inflow of afferent impulses from the pressoreceptors in the carotid sinus and the aorta. Thus, it appears that an increase in the activity of the sympathetic nervous system acts in two distinct and antagonistic ways upon the reticular formation. The arterial hypertension associated with an increased concentration of circulating epinephrine may inhibit the spinal motor neuron. This inhibition depends upon discharges originating in the pressoreceptors, which directly activate the bulbar inhibitory portion of the reticular formation. This is a reflex mechanism acting in a tonic way. The increase of concentration of circulating epinephrine also produces a facilitation of the spinal motricity which is essentially mediated by the reticular facilitatory formations localized in the anterior part of the brain stem. The fact that certain reticular neurons are sensitive to epinephrine is important in view of Vogt's observation that the hypothalamus is rich in catecholamines (mainly norepinephrine).⁷ The functional significance of the two antagonistic effects of epinephrine upon the brain stem is also important when considering the different physiological situations in which a peripheral sympathetic discharge occurs, as in emotional states, muscular effort, and various forms of physiological stress. The excitatory action of epinephrine exerting its effects upon the reticulocortical and the reticulospinal system considerably heightens the level of activity of the whole somatic system. The counterregulation exerted by the pressoreceptors acting by way of the inhibitory bulbar portion of the reticular formation keeps in check the output of the muscular apparatus and co-ordinates its activity with the capacity of the circulatory system. According to Dell and associates, this humoral co-ordination between somatic and vegetative activities discloses the existence within the nervous system of integrative processes which have a biochemical basis.⁹

Ingvar and Soderberg have studied the intimate correlation which exists between the cerebral blood flow, systemic blood pressure, and the electrical activity of the cortex of the brain as reflected by the E E G.¹⁰ They showed that arousing stimuli which activate the E E G are associated with a marked increase in the cerebral blood flow which is independent of changes in the systemic blood pressure. Spontaneous changes in cerebral blood flow accompanied by changes in the E E G were also observed to occur in the lightly anaesthetized animal. Small doses of anaesthetic agents, sufficient to induce a sleep pattern of the E E G and a block of the arousal reaction, paralyse the mechanisms regulating cerebral vasomotor tone. They also found that during deep barbiturate anaesthesia the cerebral vessels are much more constricted than under light ether anaesthesia. In general, autonomic mechanisms are very sensitive to anaesthesia, causing alterations in pupillary diameter and loss of tone of cutaneous vessels, whereas, during arousal, cutaneous vasoconstriction develops and the pupils constrict. On the other hand, autonomic mechanisms which regulate systemic blood pressure are rather insensitive to deep anaesthesia since an arousal reaction in the E E G does not appear with nociceptive stimulation even though a passive change in cerebral blood flow does occur.

Brodie and associates believe that many of the lipid soluble congeners of

norepinephrine easily penetrate the blood-brain barrier and stimulate the central adrenergic receptors of Hess^{11,12} Such phenylethylamines as amphetamine, dihydroxyphenylalanine (dopa) and lysergic acid diethylamide produce an EEG arousal pattern, enhanced psychomotor activity and an increased central sympathetic outflow¹³⁻¹⁷ Vasopressor drugs can also produce an arousal response in the intact animal during intravenous and inhalation anaesthesia¹⁸⁻¹⁹ In support of Brodie's position, the awakening effect of the phenylalanines (specifically *m*-tyrosine and dopa) following administration of reserpine has been said to be due to their eventual transformation to catecholamines^{20,21} As the stepwise transformation of tyrosine to dopa, dopamine, or tyramine and subsequently to norepinephrine and epinephrine is well established (as shown in Fig 1) and since

FIGURE 1 Some precursors of epinephrine

the central stimulating effects of some catecholamines following intravenous injection are described, one may ask the further question "Do other precursors of epinephrine produce an arousal response that can be identified by clinical observation in the intact animal which has not been subjected to other drugs or contact stimulation?"

This study was undertaken therefore to evaluate the effect of the epinephrine precursors on spontaneous activity. Tests were carried out with *l*-tyrosine, *dl-m*-tyrosine, *dl*-3,4-dihydroxyphenylalanine (dopa), 3,4-dihydroxyphenylethylamine (dopamine), levoarterenol (norepinephrine), and epinephrine.

Tests were carried out with tyramine, methyl dopate hydrochloride (Aldomet^R ester), and amphetamine sulphate, in addition, because they are closely related in chemical structure to the endogenous catecholamines. Tyramine (4-hydroxyphenylethylamine) is a product of protein autolysis which occurs in the intestine of most animals. It was used in this study because its pressor action is the same as that of norepinephrine, although its potency is only about 2 per cent by comparison. Methyl dopate acts in the body as a decarboxylase inhibitor of dopa, thereby interfering with the formation of dopamine, and hence norepinephrine and epinephrine. This is the apparent mechanism by which it may cause hypotension. It also has a slight psychomotor effect (mood depression) which is quite

distinct from the opposite effect of amphetamine—which has psychomotor actions which are similar to those of epinephrine. Amphetamine is believed to act centrally by releasing endogenous catecholamines (dopamine) and it appears to prevent the uptake of circulating norepinephrine by the tissues^{22 23} It differs from the sympathomimetic amines by having a nicotinic action, which may explain some of its central and peripheral stimulation and after-depression properties²⁴

A simple device available in most laboratories was used to permit quantitative measurements of activity and direct observation without use of electrical contacts or restraints on the rats. The effect of some of these drugs on the hypnosis produced by methohexital anaesthesia was also measured by timing the rate of induction and duration of anaesthesia.

MATERIALS AND METHODS

Experiments were performed with 693 Sprague-Dawley male rats weighing 80 to 100 grams. The rats were maintained in individual cages with dry food and tap water available *ad lib*.

FIGURE 2 Apparatus for recording the overt activity of the rat ("jigglograph")

The apparatus for recording activity is shown in Figure 2. Its basic arrangement consisted of a hypodermic needle attached beneath the receiving pan of a standard laboratory weighing scale and connected to a force displacement transducer B, which shows the details of the apparatus marked A. This led to an amplifying and recording system (Grass polygraph, P in Figure 2). After placing a rat in a transparent rigid box (plexiglas) mounted on the receiving pan of the weighing device, sensitivity adjustment of the polygraph permitted even the smallest jiggling of the scale, such as would be caused by a panting movement, to be recorded.

In order to quantitate the record, the number of deflections were measured on the recording paper and counted in the following four categories: 5 mm (deflection length range, 4–9 mm), 10 mm (10–14 mm.), 15 mm (15–19 mm), and 20 mm (20 mm or more), and the total amount of the deflections in centimetres was recorded as an activity score for the observation period.

Activity

Individual one-hour recordings, which included an initial 20-minute control period and a 40-minute period after the drug injections, were taken with 60 rats under the following 15 conditions (four rats per condition): (1) no drug, (2) 0.9% saline (20 ml/kg), (3) *l*-tyrosine (200 mg/kg), (4) *l*-tyrosine (600 mg/kg), (5) *dl-m*-tyrosine (200 mg/kg), (6) *dl-m*-tyrosine (600 mg/kg), (7) dopa (200 mg/kg), (8) dopa (600 mg/kg), (9) dopamine (200 mg/kg), (10) dopamine (600 mg/kg), (11) methyl dopate (200 mg/kg), (12) methyl dopate (600 mg/kg), (13) tyramine (200 mg/kg), (14) tyramine (600 mg/kg), and (15) amphetamine (10 mg/kg). All drugs were given by intraperitoneal injection. Two of each set of four rats were later sacrificed and the peritoneum was examined to ensure that no irritation had occurred. Recorded activity (in cm deflections) was tabulated for the 10 successive four-minute time intervals, starting 20 minutes after the drug injection.

Hypnosis

To evaluate the effect of the drugs on the duration of anaesthesia, first 106 rats (control group) were injected intraperitoneally with methohexital (0.5% solution, 30 mg/kg). Induction time (the time from injection to loss of righting reflex and loss of consciousness) and maintenance time (the time from the loss of consciousness until the animal recovered enough to raise its head and walk) were recorded in minutes.

An additional 527 rats were randomly divided into 12 groups (see Table I). There were eight test drug groups (33–35 rats per group) and four control groups (58–69 rats per group). The rats in all groups were anaesthetized with methohexital as in the control test group and anaesthesia induction and maintenance times were recorded. One week later, all groups were again anaesthetized with the same dose of methohexital. The four control groups were injected with 0.9 per cent saline (20 ml/kg) simultaneously whereas the eight test groups each received one of four drugs by two methods as follows. Method 1: intraperitoneal injection of the drugs 20 minutes prior to administration of methohexital to

TABLE I
DESIGN OF EXPERIMENT TO COMPARE EFFECT OF DRUGS ON HYPNOSIS IN
RATS PRODUCED BY METHOHEXITAL (30 mg /kg , i p)

Drugs (600 mg /kg , i p)	No. of controls	Drug test groups	
		Method I*	Method II†
No drug	106	—	—
<i>m</i> -Tyrosine	58	35	35
Dopa	63	33	33
Methyldopate	66	33	33
Amphetamine	69	34	35

*Method I — Test drug was injected 20 minutes *prior* to the administration of methohexital

†Method II— Test drug was injected immediately following the administration of methohexital

observe the duration of anaesthesia while the effect of the test drug motor activity was at its peak (four groups) Method 2 intraperitoneal injection of drugs *immediately following* that of methohexital, to see whether the response was different (four groups) For each rat, anaesthesia induction and maintenance times were recorded in minutes as described above *m*-Tyrosine, dopa, and methyldopate were given in a dose of 600 mg /kg , whereas 10 mg./kg amphetamine was given

RESULTS

Activity

When each rat was placed in the transparent chamber, a considerable amount of exploratory activity was noted at first, then the activity subsided and the rat usually appeared to be asleep Part A of Figure 3 shows the tracings representing exploratory activity and periods of quiescence as recorded on the "jigglograph" Part B of Figure 3 shows the typical records of the activity following injection of various catecholamine precursors and the non-physiological chemicals *m*-tyrosine, methyldopate, and amphetamine at the time when maximum activity was observed This maximum activity was invariably observed during the 20 to 60 minutes following the drug injections The latent period of about 20 minutes prior to the manifestation of drug action probably represents the time required to produce a motor response directly or to convert these drugs in the body to some physiologically active form

Figure 4 shows the graphic representation of the activity counts per 4-minute time interval over the 40-minute period starting from 20 minutes following the injections of the various drugs Each line shows the typical response for one representative experiment with the designated drug Table II shows the detailed results obtained with four individual rats for each of the drugs given in doses of 200 mg and 600 mg per kg of body weight

It may be seen in Figure 4 and Table II that the rats injected with the non-physiological compound *m*-tyrosine (200 and 600 mg /kg) were somewhat more active than those given the natural *l*-tyrosine While 200 mg /kg of dopa had

FIGURE 3 Jigglograph tracings before (part A) and after (part B) administration of various drugs

Rat Activity Counts from Jigglograph Recording in cm from 20 to 60 min after Injection of Drugs

FIGURE 4 Rat activity counts from jigglograph recording in cm from 20 to 60 minutes after injection of drugs

TABLE II

RAT ACTIVITY COUNTS PRODUCED BY THE INTRAPERITONEAL INJECTION OF VARIOUS DRUGS

Drugs		Rats				Mean	Standard deviation
		1	2	3	4		
1	No drug	25 6	15 6	10 5	20 6	18 1	6 5
2	0.9% saline 20 ml /kg	10 5	21 5	5 9	10 1	12 0	6 6
3	L-Tyrosine 200 mg /kg	24 4	34 9	35 7	19 0	28 7	7 8
4	L-Tyrosine 600 mg /kg	370 0	397 5	440 1	357 5	391 3	36 6
5	m-Tyrosine 200 mg /kg	197 0	207 7	257 3	178 9	210 2	33 5
6	m-Tyrosine 600 mg /kg	458 0	478 8	547 9	393 9	469 7	63 5
7	Dopa 200 mg /kg	40 0	56 7	36 9	46 6	45 0	8 7
8	Dopa 600 mg /kg	2615 0	3156 6	2315 4	2173 2	2565 0	435 2
9	Dopamine 200 mg /kg	79 0	95 8	59 4	63 9	74 5	16 4
10	Dopamine 600 mg /kg	122 5	177 5	98 3	201 7	150 0	47 8
11	Methyl dopate 200 mg /kg	0	0	0	0	0	0
12	Methyl dopate 600 mg /kg	103 5	115 3	90 5	165 3	118 7	32 7
13	Tyramine 200 mg /kg	181 7	128 7	178 2	220 4	177 2	37 6
14	Tyramine 600 mg /kg	115 0	128 7	198 3	202 3	161 0	45 7
15	Amphetamine 10 mg /kg	2317 6	2986 9	2014 7	2437 5	2439 2	405 6

little effect, 600 mg/kg produced some of the highest activity levels recorded among the drugs tested. Dopamine had some effect at 200 mg/kg, but very little compared to that of dopa at 600 mg/kg. Methyldopate appeared to cause total depression at the 200 mg/kg dose, but produced a little stimulation with the 600 mg/kg dose. The effect of tyramine was similar to that of dopamine but neither drug caused an increase in activity with an increase in dosage. The effect of amphetamine (10 mg/kg) was similar to that of 600 mg/kg dopa. Injection of a tolerable single maximum dose of epinephrine and norepinephrine (2 mg/kg) produced very limited activity changes, the transient effect being due to their evanescent action.

Although activity counts increased following the injections of tyrosine, tyramine, and dopamine, in comparison with the control tests with and without saline, these drugs seemed to produce a peculiar sedative state characterized by partially or completely closed eyes, immobility, and the general appearance of sleep. Numerous aperiodic startle responses were observed in these virtually dormant animals, which accounts for the augmented activity values derived from the recording.

Rats given 10 mg/kg amphetamine or 600 mg/kg of dopa did not react in this manner. *Instead, they showed a continuous state of agitation.* With 200 mg/kg dopa, the rats showed only a transient increase in recorded activity, but no sedative effect was apparent.

Direct visual observations of the rats indicated rather obvious differences in the response to dopa compared with that to amphetamine. Dopa seemed to produce purposeful and co-ordinated motor movements whereas, in addition to co-ordinated motion, amphetamine produced a constant tremor-like movement of the extremities and facial musculature.

Hypnosis

Mean induction and maintenance times in minutes, under the control and the two-test procedure conditions, and the probability values obtained with the Fisher "*t*" test are shown in Table III. Mean induction times (10 to 16 minutes) and maintenance times (13 to 20 minutes) of the rats anaesthetized with methohexital without saline (106 rats) and with saline (527 rats) were similar. It was assumed therefore that the control tests provided a valid base-line with which to compare the response to the combinations of methohexital with *m*-tyrosine, dopa, methyldopate, and amphetamine.

Induction times were longer with all the test drugs except amphetamine in the first method, while in the second method (simultaneous injection of hypnotics and precursors), *m*-tyrosine and dopa appreciably prolonged the induction time. It may be seen also that with the first method the maintenance time was significantly prolonged with *m*-tyrosine, dopa, and methyldopate, whereas amphetamine did not prolong the maintenance time. Similarly, when the precursors were given at the same time as the anaesthetic, maintenance times were prolonged in comparison with the controls, with the exception of amphetamine, which tended to shorten anaesthesia time.

TABLE III
SUMMARY OF DATA SHOWING EFFECT OF DRUGS ON DURATION OF METHOHEXITAL ANAESTHESIA

Drugs	Time of injection*	Control groups, mean time (minutes)	Experimental groups, mean time (minutes)	Significance (<i>p</i> values)
<i>m</i> -Tyrosine 600 mg /kg , i p	I IT†	1 0 ± 0 37	1 9 ± 0 76	>0 001
	‡MT‡	13 8 ± 4 3	25 2 ± 10 0	0 02-0 001
	II IT	1 1 ± 0 33	1 6 ± 0 52	>0 001
	MT	13 6 ± 4 6	19 4 ± 7 0	N S
Dopa 600 mg /kg , i p	I IT	1 2 ± 0 13	2 9 ± 0 15	>0 001
	MT	17 4 ± 6 5	35 7 ± 15 0	>0 02
	II IT	1 3 ± 0 28	2 1 ± 0 72	>0 001
	MT	15 1 ± 3 1	24 8 ± 9 3	>0 02
Methyldopate 600 mg /kg , i p	I IT	1 4 ± 0 40	2 3 ± 0 83	>0 001
	MT	17 3 ± 4 7	29 8 ± 9 5	>0 02
	II IT	1 3 ± 0 43	1 4 ± 0 38	N S
	MT	15 5 ± 5 2	20 1 ± 6 2	0 02-0 05
Amphetamine 10 mg /kg , i p	I IT	1 6 ± 0 40	1 3 ± 0 35	0 05
	MT	19 2 ± 6 6	20 9 ± 6 0	N S
	II IT	1 3 ± 0 24	1 6 ± 0 46	0 05
	MT	19 8 ± 6 9	17 8 ± 6 0	N S

*Time of injection I = drugs injected 20 minutes before methohexital
II = drugs injected immediately after methohexital

†IT = Induction time

‡MT = Maintenance time

DISCUSSION

One of the major stumbling blocks that the clinician faces in the evaluation of overt reactions to drugs can be attributed to the lack of reliable objective criteria for defining inhibitory (or depressive) and excitatory (or awakening) responses. Any particular observed overt response can be accounted for by invoking an excess of inhibition or a deficit of excitation and a decrement of inhibition or an excess of excitation. It becomes a complex task to sort out the relative contribution and the degree of independence of these two processes. In broad psychological terms, depression or inhibition may refer to a process in the central nervous system which interferes with the ongoing perceptual, cognitive, and motor activities of the organism. The types of interference have been called temporal and spatial inhibition. Temporal inhibition is a fatigue-like state which interferes with performance and usually dissipates during rest. For instance, whenever a long-continued and monotonous visual or auditory activity is carried out, performance is adequate at first, but vigilance declines after awhile, and ceases finally on account of temporal inhibition. Spatial inhibition, on the other hand, is a process in which some form of sensory deprivation occurs, or the stimulus becomes imperceptible because another sensation has been evoked by simultaneous stimulation elsewhere in the sensory field.

In the same terms of reference, excitation or arousal refers to a process within the central nervous system which facilitates the ongoing perceptual, cognitive,

and motor activities of the organism. Sensory thresholds may be influenced by the organism's degree of central nervous system excitation in addition to peripheral nervous factors and external noxious stimuli.²⁵

When an animal is situated in a quiet environment and is virtually free of all extrinsic sensory impulses, its electroencephalogram discloses alterations from a more or less alert state to one of sleepiness. An alert pattern is readily evoked by any peripheral external sensory stimulus, and the EEG shows widespread alert activity over the cortex, especially when the animal is touched or a painful stimulus is applied. Himwich has posed the question: Does epinephrine cause arousal in such an animal by direct action on the reticular formation, or is the effect the result of stimuli originating from peripheral receptors that are activated by epinephrine as occurs when the animal is touched?²¹ The work reported by the French workers cited above⁹ indicates that epinephrine exerts a direct alerting effect upon the reticular formation and that the intrinsic mechanism is adrenergic in character. They showed that the alerting effect of epinephrine is *not* due to its vasopressor action because there were discrepancies in the time course of the blood pressure changes and EEG alerting. These observations have been clearly confirmed by others.^{26,27} However, we have directly observed overt alerting responses in dogs immediately following the injection of various vasopressors during moderately deep anaesthesia,^{18,19} so it seems that in the intact organism epinephrine and related vasoactive substances stimulate a wide range of receptors and the final psychic effect is the result of numerous cardiovascular, respiratory, and cortical effects, including stimulation of baroreceptors (which might cause *inhibition* of the reticular formation), but at the same time, or preceding this, local cerebrovascular changes occur which can alter cerebral blood flow and cause an overriding *stimulation* of the reticular formation.^{10,28} Thus, the arousing effect of epinephrine can be attributed to changes in cerebral blood flow even if appreciable or immediate hypertension does not occur. Capon has shown that epinephrine antagonists inhibit central activation to the same extent that they prevent the cardiovascular effects of epinephrine, and has observed, as we have, that other vasopressors can cause arousal.²⁹ In a review of the effect of various drugs on the cerebral circulation, Sokoloff cites work which showed that the intravenous administration of epinephrine and amphetamine has little or no effect on cerebral blood flow, whereas norepinephrine and several of the vasopressor sympathomimetic drugs do produce an appreciable quantitative increase in the cerebral blood flow—which may reflect only a passive response of the cerebral circulation to a rise in systemic blood pressure and/or the effects of an increased cerebral metabolic rate.³⁰

It is evident that the constant stream of sensory and proprioceptive stimuli, to which an awake animal is exposed all day, sets up inhibitions of the stimulus-produced kind which have no time to dissipate unless it is placed in a confined area where external stimulation is eliminated. One can also imagine a constitutional property of the central nervous system such that an animal is constantly in a state of greater or lesser excitation or inhibition while awake. Drugs which affect the sensorium, the cardiovascular system, and the respiratory system can undoubtedly alter this sensory state. From a physiological point of view, we know

that changes in the excitatory or arousal state of the central nervous system and the reticular system can be monitored by continuously recording the electrical activity in the brain (E E G), neuromuscular action potentials (E M G), and galvanic skin resistance (G S R). It appears obvious, therefore, that any alteration in spontaneous activity must be due to widespread effects rather than one confined to the brain. We chose the use of the apparatus described in this report in order to eliminate all forms of contact which might modify the response of the rats to the drugs tested.

The important lesson we learned from this study was the fact that mechanical recording of spontaneous activity does not alone reveal the entire overt behavioural responses of the intact experimental animal, so it is of the utmost importance to observe the animal directly visually and to annotate the responses before, and while it is under the influence of drugs, if one desires reliable information as to overt responses.

Previous work that has indicated an alerting effect by derivatives of phenylalanine (tyrosine, dopa, dopamine, and noradrenaline)^{20 21,31 32 33} for the most part was done in animals that were tranquillized by large doses of reserpine or prepared with monamine oxidase inhibitors. Carlsson and associates found that dopa is capable of penetrating into the brain via the blood. It is then rapidly decarboxylated to form dopamine, and the dopamine thus formed is concentrated in those parts of the brain where either dopamine or norepinephrine is present normally.³¹ They found that if the catecholamine level in the brain was increased by the injection of dopa, central excitation was observed. The exciting effect was greater if the animal was previously depressed by monamine oxidase. The loss of alertness and motor activity following reserpinization was restored also by administration of dopa. These observations seem to support the view that catecholamines in the brain are involved in the control of psychomotor activity.

The rather striking difference between the effects of amphetamine and dopa on spontaneous activity that we observed directly and the visual observation of startle movements during a sleep-like state following administration of tyrosine raises doubts as to the validity of the idea that lipid-soluble congeners of epinephrine easily penetrate the blood-brain barrier and cause the same kind of stimulation in the central adrenergic receptors of Hess's ergotropic division.^{11 12} If these phenylethylamines produce an increase in sympathetic activity by a mechanism similar to that produced by norepinephrine and epinephrine—as might be expected because their chemical structures are closely related—their lipid solubilities are comparable, and because they can undergo a stepwise transformation towards catecholamines, then they should produce a more obvious and an essentially similar activity also. The "jugglograph" recordings, together with our direct observation that there was a lack of dose-response relationships following administration of dopamine, tyramine, and related drugs, did not indicate to us that there was any such effect.

The effect of the short-acting hypnotic (methohexital) combined with the catecholamine precursors also did not support the "similar mechanism" position, cited above, because administration of these catecholamine precursors (except amphetamine) definitely *prolonged* maintenance time of anaesthesia. The effect

on induction time by amphetamine with Method 1 (but not with Method 2) seems to indicate that this drug produces some physiological or psychological effect on the awake rat which causes a shortening of induction time when it is injected prior to methohexital. This may be attributed to physical exhaustion due to drug-induced excitement which would not occur when the amphetamine is injected at the same time as the anaesthetic. If these phenylethylamines all act upon the same receptor site for sympathetic stimulation and in the same manner, then these drugs should have shortened the hypnotic effect of methohexital, as amphetamine may do, instead of uniformly prolonging it. Since amphetamine was the only agent tested that did not prolong the maintenance time of anaesthesia, it does not seem reasonable to classify other catecholamine precursors (such as dopa) with amphetamine as a sympathetic stimulating agent which can increase psychomotor activity. Indeed, the effect of the precursors we studied on the central nervous system seemed quite different.

Another worker has shown that there is a decrease of reflex activity of rats with dopamine whereas amphetamine increases it.³⁴ Smith and Dews also reported that dopa markedly suppressed the locomotor activity of normal rats.³⁵ Randrup and associates reported that amphetamine failed to be antagonized by adrenergic blocking agents and, therefore, is not an adrenergic drug.³⁶ These results seem to support our observations, which indicate that the effects of amphetamine and the phenylalanine derivatives cannot be considered in the same category.

A possible explanation for the observed prolongation of hypnosis with methohexital by some of the catecholamine precursors can be drawn from the study by Conney and associates, who demonstrated their depressant action on enzymes in the liver microsomes which metabolize barbiturate compounds.³⁷ We can therefore assume that the catecholamine precursors may upset the metabolism of methohexital in the liver enough to prolong its anaesthetic effect. The catecholamine precursors may also prolong hypnosis by reducing the rate of elimination of barbiturates through the kidneys. Welles and associates reported that 80 per cent of methohexital given to rats was excreted in faeces while 20 per cent was excreted in urine,³⁸ which indicates that both of the above assumptions may be valid. One can surmise also that epinephrine precursors might prolong the effect of barbiturate anaesthesia through a direct depressant effect on the brain itself.

SUMMARY AND CONCLUSIONS

Adrenergic arousal reactions have been shown to occur by behavioural responses as well as by changes in the electroencephalogram due to stimulation of the ascending sensory receptors in the reticular activating system. There is good evidence that the arousal response is not due to the vasopressor action of epinephrine, although clinical observations with most vasopressor drugs point to such an explanation. Local vascular changes in the brain which are not necessarily reflected in the general circulation have been suggested and demonstrated as a likely explanation for psychomotor activation by epinephrine.

The precursors of epinephrine also seem to play a role in the control of alertness and motor functions. The negative aspect of this idea explains in part the decrease

in spontaneous activity and depression of psychomotor activity in response to reserpine administration. The question of whether or not phenylalanine derivatives can themselves produce awakening reactions has been studied to a limited extent. A very large dose of *l*-dopa does produce an awakening response in reserpinized mice. To a less extent, *m*-tyrosine appears to have a similar effect. The two isomers of dopa and *o*-tyrosine, however, are devoid of the activating property. Other epinephrine precursors, except norepinephrine, are virtually inert.

This study was devised to determine whether the epinephrine precursors (phenylalanine derivatives) as well as tyramine, methyldopate, and amphetamine produce an alerting response and increased motor activity in rats deprived of extrinsic stimulation. Before and following intraperitoneal injection of the test drugs, rats were placed individually in a transparent box that was mounted on a weighing scale and attached to a recording apparatus which inscribed on paper even the slightest movement of the rat. All of the catecholamine precursors studied produced some alterations in the spontaneous activity of the rats. *dl*-Dopa caused the greatest increase in motor activity, the reaction being quantitatively similar to that seen with amphetamine. *l*-Tyrosine, *m*-tyrosine, tyramine, and dopamine produced a peculiar effect on the rats—they appeared as if they were under the influence of a potent sedative, but they had numerous aperiodic startle movements which, on the recording, appeared as if they had a substantial alerting reaction. Although the activity tracing for amphetamine and dopa appeared similar, the rats appeared quite different on direct observation. Dopa produced an increase in motor activity which appeared co-ordinated and purposeful, whereas amphetamine caused an increase in co-ordinated movements but there was also a generalized increase in body movement that resembled muscle trembling, similar to that seen in nicotine poisoning.

The effect of *m*-tyrosine, dopa, methyldopate, and amphetamine given 20 minutes before or simultaneously with methohexital anaesthesia revealed, contrary to expectation, that hypnosis was uniformly prolonged with each, except with amphetamine. These data were interpreted as contradicting the opinion that the psychomotor effects of the catecholamine precursors can be considered in the same category as epinephrine. It was also evident that amphetamine acts quite differently from the precursors of epinephrine, whereas methyldopate either suppresses motor activity or has very little activity in this regard.

RÉSUMÉ

Des réactions adrénérgiques de réveil se sont manifestées aussi bien par les réponses des sujets que par les modifications sur le tracé électroencephalographique à la suite de la stimulation des récepteurs sensoriels ascendants dans le système réticulé activateur. Il existe une évidence assez forte que la réponse d'éveil n'est pas due à l'action vasopressive de l'épinephrine bien que des observations cliniques en employant la plupart des médicaments vasopresseurs portent à croire cette explication. On a suggéré et on a démontré, comme une explication vraisemblable de l'activation psychomotrice par l'épinephrine, des changements qui n'ont pas nécessairement d'écho sur la grande circulation.

Même les précurseurs de l'épinéphrine semblent jouer un rôle dans la contrôle

des fonctions de veille et de motricité. L'aspect négatif de cette idée explique en partie la diminution de l'activité spontanée et la dépression de l'activité psychomotrice en réponse au traitement par la réserpine. Jusqu'à un certain point, nous avons essayé de déterminer si oui ou non les dérivés de la phénylalanine pouvaient eux-mêmes provoquer des réactions de réveil. Une dose très forte de *l*-dopa est capable de provoquer une réponse de réveil chez la souris traitée à la réserpine. De façon moins marquée, la *m*-tyrosine semble provoquer une réponse semblable. Les deux isomères de dopa et *o*-tyrosine, toutefois, sont dépourvus de toute propriété d'activation. Les autres précurseurs de l'épinephrine, à l'exception de la norépinephrine, sont virtuellement mortes.

Nous avons entrepris cette étude dans le but de préciser si les précurseurs de l'épinephrine (les dérivés de la phénylalanine) et la tyramine, le dopate de méthyle et l'amphétamine peuvent provoquer des réponses de réveil et une activité motrice accrue chez des rats dépourvus de stimulation extérieure.

Avant et après l'injection intrapéritonéale à des rats des médicaments étudiés, nous avons placés ces animaux séparément, chacun dans une boîte transparente, montée sur une balance et attachée à un appareil enregistreur qui inscrit sur un papier le plus léger mouvement du rat. Tous les précurseurs de catécholamine étudiés ont produit certaines modifications de l'activité spontanée des rats. La dopa a produit la plus grande augmentation de l'activité motrice, mais, quantitativement, la réaction a été semblable à celle observée avec l'amphétamine. *l*-Tyrosine, *m*-tyrosine, la tyromine et la dopamine ont produit, sur les rats, un effet particulier, ils semblaient devenir sous l'effet d'un puissant sédatif, mais ils faisaient de nombreux mouvements à intervalle irrégulier, sur le tracé, comme s'ils avaient une forte réaction d'éveil.

Bien que les tracés de l'activité pour l'amphétamine et la dopa soient semblables, à l'observation directe, les rats semblaient tout différents. La dopa a produit une augmentation de l'activité motrice qui a semblé coordonnée et intentionnée, tandis que l'amphétamine a produit une augmentation des mouvements coordonnés, mais il est apparu également une augmentation totale des mouvements du corps qui ressemblait à un tremblement musculaire, comparable à ce que nous observons au cours de l'empoisonnement par la nicotine. L'effet de l'*m*-tyrosine, de la dopa, du dopate de méthyle et de l'amphétamine donnés 20 minutes avant ou en même temps qu'une anesthésie au méthohexital a montré que, contrairement à ce que nous étions en droit d'attendre, avec chacun de ces médicaments, le sommeil a été uniformément prolongé à l'exception de l'amphétamine.

Ces résultats sont interprétés comme contredisant l'opinion voulant que les effets psychomoteurs des précurseurs des catécholamines soient considérés de la même catégorie que ceux de l'épinephrine. Il était également manifeste que l'amphétamine, agit tout à fait différemment des précurseurs de l'épinephrine alors que le dopate de méthyle peut, soit supprimer l'activité motrice soit n'agir que très peu dans ce sens.

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