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ACTIONS OF CERTAIN AMINES ON CEREBRAL CORTICAL NEURONES

BY

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A number of derivatives of tryptamine and phenethylamine, and certain other compounds, were tested on neurones in the cerebral cortex of cats by iontophoretic release from micro-pipettes. The characteristic action of many of these compounds was a depression of the neuronal discharge initiated by synaptic activity or by the application of L-glutamate; imidazolylacetic acid, dopamine, ephedrine and ergometrine were particularly effective. Catechol amines, hydroxytryptamines and imidazolylacetic acid had a relatively quick and rapidly reversible action, not unlike that of γ-aminobutyric acid, whereas ephedrine and derivatives of lysergic acid diethylamide caused a slower and more prolonged depression of the amplitude of spikes, rather like atropine. Several compounds, including 5-hydroxytryptamine, adrenaline and ergometrine, could also excite the same neurone when larger amounts were applied. A few substances, such as dopa and methylergometrine, had a predominantly excitant action.

There has been much speculation during the course of the last decade concerning the significance of several indole and catechol amines which occur naturally in the brain. Interest in these substances has increased as a result of recent developments in the study of psychotropic compounds, some of which are structurally related to the indole and catechol amines (lysergic acid diethylamide, bufotenine, mescaline and others).

Evaluation of the importance of these compounds in central nervous mechanisms and definition of their sites of action have been hindered by rather imprecise techniques of administration. The intravascular route is associated with several difficulties including the impermeability of the blood–brain barrier, stimulation of peripheral receptors and alteration of the blood supply to the brain. Furthermore, different neurones may not respond in a similar fashion and the behaviour of neurones in any one area of the brain may be modified as a result of changes in neuronal activity elsewhere. However, in spite of these limitations, many of these compounds have been shown to depress the responses evoked transcallosally in the cerebral cortex (Marrazzi & Hart, 1955; Marrazzi, 1957). These findings were substantiated by Ochs, Booker & Aprison (1960), who showed that 5-hydroxytryptamine, applied topically, depressed cortical responses to direct stimulation;

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Grenell (1957) has reported that lysergic acid diethylamide depressed evoked responses when injected into pial blood vessels.

Since compounds such as 5-hydroxytryptamine, adrenaline, noradrenaline, dopamine and histamine occur in small but consistent amounts in the cerebral cortex (Amin, Crawford & Gaddum, 1954; Vogt, 1954; Bogdanski, Weissbach & Udenfriend, 1957; Bertler & Rosengren, 1959; Carlsson, 1959; Adam, 1961) together with the enzymes responsible for their synthesis and destruction, it was of interest to test their actions on individual cortical neurones by iontophoretic application from micropipettes (Curtis & Eccles, 1958; Curtis, Phillis & Watkins, 1959, 1960; Krnjević & Phillis, 1961, 1963a, b). A series of related catechol and indole amines and certain derivatives of lysergic acid were therefore released in the cerebral cortex by this technique. Some preliminary results have already been published (Phillis & Krnjević, 1962; Krnjević & Phillis, 1963a).

A paper has appeared recently describing the actions of compounds of a similar series on synaptic transmission in the lateral geniculate nucleus (Curtis & Davis, 1962). Although similar techniques were employed in both investigations, the results and conclusions differ in some important respects and it is apparent that neurones in various regions of the brain may have remarkably different properties.

METHODS

Experiments were performed on cats anaesthetized usually with intraperitoneal allobarbitone (70 mg/kg; Dial compound, Ciba), but sometimes ether or chloralose (80 mg/kg) were used instead; the more important and active compounds were also tested on neurones in the unanaesthetized "cerveau isolé" of the cat to eliminate any possible interference by anaesthetics. A wide exposure of one cortical hemisphere was made to allow recording from different regions and to reduce the pulsations of the brain. The surface of the cortex was covered with a layer of transparent polyethylene to prevent drying and with a second layer of black polyethylene to minimize cooling, a small opening being left for the insertion of the microelectrode. A small Perspex disc was usually applied to the surface of the brain to reduce respiratory and vascular pulsations (Phillips, 1956), the microelectrode passing through a hole in its centre. The temperature of the animal was maintained at $37 \pm 1°$ C by an automatically controlled heating pad under its abdomen (Krnjević & Mitchell, 1961).

The construction and filling of the multi-barrelled micropipettes have already been described in detail (Krnjević & Phillis, 1963a). Most of the compounds used during this investigation are in their cationic form at a neutral *p*H (Albert, 1952; Lewis, 1954; Vane, 1959; Tuckerman, Mayer & Nachod, 1959; Hill & Usherwood, 1961) but the solutions were usually kept at a *p*H of about 4 to 5 to improve their stability. The pipettes were stored in the dark at 4° C during the 48 hr required for equilibration. Drugs were then passed as cations from these solutions. L-Glutamate was always present in one barrel of each pipette and was passed as an anion from solutions at *p*H 8.

Each compound was released from at least two different micropipettes, testing a total of not less than five neurones; the majority of compounds were tested on more than ten neurones, mostly in the pericruciate region.

Peripheral afferent volleys were evoked by electrical pulses of 0.1 msec duration, at an intensity of 1 to 10 V and a frequency of about 0.5 shocks/sec, led through stainless steel needles inserted into the extremities of the limbs. The actions of some of the compounds were also tested on the antidromic spikes of corticospinal neurones (Betz cells). In these instances the medullary pyramidal tracts were exposed and stimulated with bipolar needle electrodes (Phillips, 1956; Krnjević & Phillis, 1963b).

The following firms and individuals supplied the various drugs used. Those marked with an asterisk sent the drugs as free gifts, for which we are very grateful. *Dr. A. Cerletti (Sandoz): 4-hydroxytryptamine oxalate, 6-hydroxytryptamine creatinine sulphate, DL-6-hydroxytryptophan, 7-hydroxytryptamine, 4-hydroxy-*NN*-dimethyltryptamine, 4-methoxytryptamine, 12-hydroxyergometrine bimaleate, D-lysergic acid diethylamide tartrate, methylergometrine and methysergide. Light & Co.: L-dopa, harmine hydrochloride, DL-4-hydroxytryptophan, 5-hydroxytryptamine bimaleinate, DL-5-hydroxytryptophan, 5-hydroxy-*NN*-dimethyltryptamine, mescaline sulphate, 5-methoxytryptamine and tyramine hydrochloride. British Drug Houses: adrenaline acid tartrate, (±)-amphetamine sulphate, (−)-ephedrine, ergometrine acid maleate, histamine acid phosphate, (−)-noradrenaline, DL-tyrosine, tryptamine hydrochloride and DL-tryptophan. *Dr J. R. Vane: (−)- and (+)-ephedrine, (−)- and (+)-pseudoephedrine, *N*-methylephedrine, norephedrine and norpseudoephedrine. California Corporation for Biochemical Research: *NN*-diethyltryptamine, dopamine hydrochloride, L-histidine, imidazolyl acetic acid, indol-3-ylacetic acid and yohimbine hydrochloride. May & Baker: 5-hydroxytryptamine creatinine sulphate and chlorpromazine. Eli Lilly & Co.: isoprenaline sulphate and dichlorisoprenaline. *Regis Chemical Co.: *NN*-dimethyltryptamine and melatonin. *Dr H. McLennan: dopamine hydrochloride. Pabst Laboratories: adenosine triphosphate (sodium salt). Hopkin & Williams: DL-phenylalanine.

RESULTS

Section I

It is our aim in this section of the paper to describe in detail the actions of representatives of the major groups of compounds tested. Section II of the paper will then outline the properties of the other compounds which were selected either on the basis of their structural relationship to these representative substances or because of their psychotropic properties.

Dopamine was selected as the representative of the phenethylamine derivatives, a group which includes several compounds with actions on the central nervous system, such as adrenaline, noradrenaline, amphetamine and mescaline (Carlsson, 1959; Rothballer, 1959). In view of its potency and short duration of action, dopamine was used as the standard for comparing the activity of compounds within the different groups.

5-Hydroxytryptamine was taken as the representative of the simple indole derivatives. Depressant actions of 5-hydroxytryptamine, when applied topically or intra-arterially to the cerebral cortex (Marrazzi & Hart, 1955; Malcolm, 1958; Ochs *et al.*, 1960) or by iontophoretic injection onto neurones in the lateral geniculate nucleus (Curtis & Davis, 1962), have been reported, although apparently the drug has no effect on neurones in the brain stem and spinal cord (Curtis & Koizumi, 1961; Curtis, 1962).

The third major group of compounds consisted of derivatives of lysergic acid, which also contains an indole nucleus. The group representative, lysergic acid diethylamide, has been widely investigated since the demonstration of its hallucinogenic properties (Stoll, 1947).

In the present investigation, compounds in each of the three groups depressed neuronal responses both to synaptic excitation and to that caused by L-glutamic acid, as well as spontaneous activity. Similar effects were observed when using different anaesthetic agents and in the unanaesthetized "cerveaux isolés." The

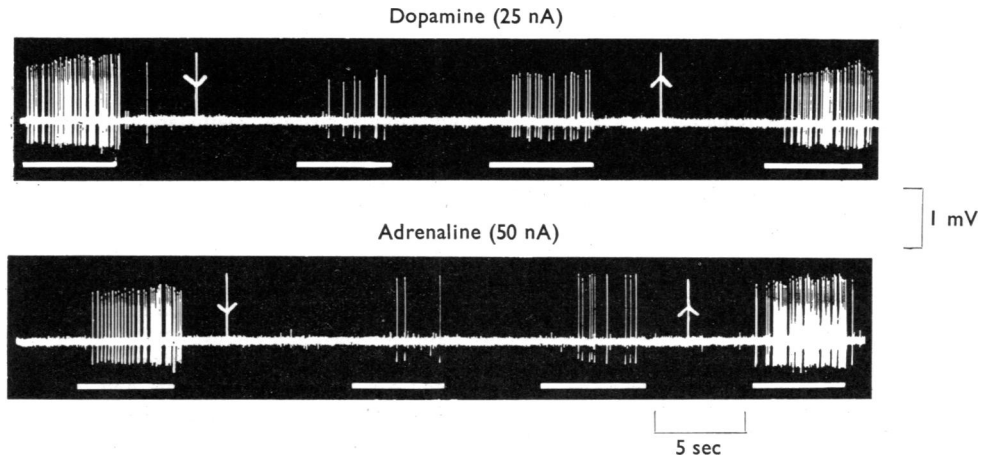

Fig. 1. Comparison of the blocking action of dopamine and of adrenaline on the excitation of a cortical unit by L-glutamate (30 nA); applications of glutamate are shown by the horizontal white lines below traces. The catechol amines were released from other barrels of same micropipette during the periods between arrows. The cat was anaesthetized with allobarbitone.

actions of dopamine and of adrenaline are shown in Fig. 1. This neurone was readily excited by L-glutamate (Krnjević & Phillis, 1961, 1963a) released at the time indicated by white lines below the traces. When examined during the simultaneous release of dopamine from another barrel of the same pipette, the effect of glutamate was considerably reduced. The application of adrenaline produced a similar change in excitability but, for adrenaline, the current had to be increased to 50 nA to obtain a depression comparable with that caused by dopamine. The actions of dopamine and adrenaline differed in another respect, for whereas dopamine caused a progressively more powerful depression when applied with larger currents, greater amounts

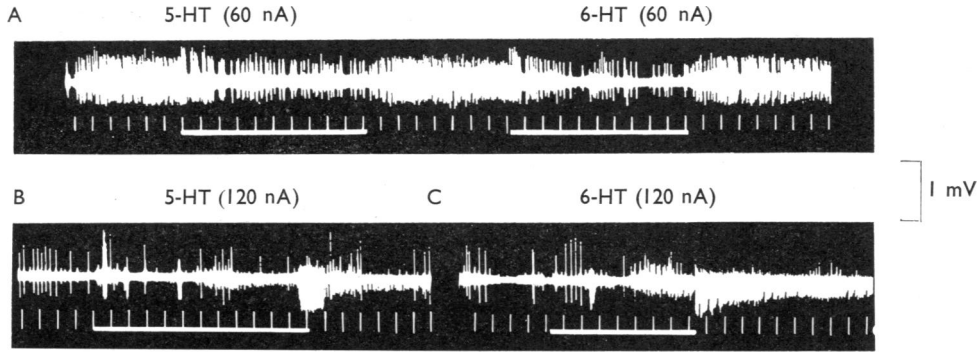

Fig. 2. A: cortical unit excited by continuous background release of L-glutamate (60 nA) from one barrel of a multibarrelled micropipette; firing was depressed by an additional release of 5-hydroxytryptamine (5-HT) or 6-hydroxytryptamine (6-HT), both at 60 nA, from other barrels, at times shown by the white lines below trace. B and C: faster release of 5-hydroxytryptamine or 6-hydroxytryptamine (at 120 nA) at the same point caused some excitation. The cat was anaesthetized with allobarbitone. Time marks, 1 sec.

of adrenaline frequently caused a delayed excitation with the cell discharging at a high frequency. This latter action was like that seen when large amounts of 5-hydroxytryptamine were applied (Fig. 2B).

5-Hydroxytryptamine had a similar action on the responses of cortical neurones initiated by glutamate. The continuous discharge illustrated in Fig. 2A was caused by a steady background release of glutamate. The discharge was depressed by the application of 5-hydroxytryptamine and even more effectively by 6-hydroxytryptamine. Larger doses of both 5-hydroxytryptamine and 6-hydroxytryptamine resulted

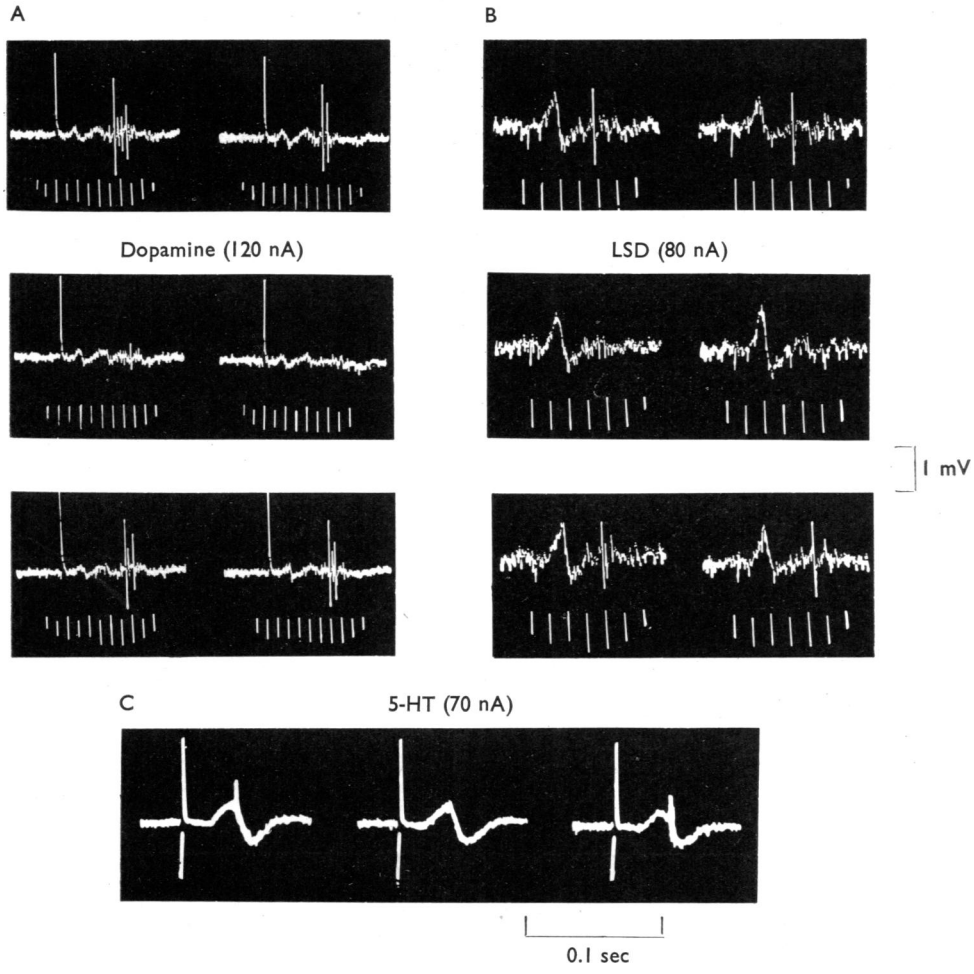

Fig. 3. Different unit responses evoked in the somatosensory cortex by peripheral stimulation, and blocked by iontophoretic release of dopamine, lysergic acid diethylamide (LSD) and 5-hydroxytryptamine (5-HT). In each case, either one (C) or two (A and B) records are shown before, during and after recovery from block, but each record in series C consists of several traces superimposed. In A, the response was obtained by stimulating electrically the contralateral hindpaw; in B and C, the contralateral forepaw. A and B were in a cat anaesthetized with chloralose, C in a cat with allobarbitone. Time marks in A and B, 10 msec.

in a delayed but fast discharge (Fig. 2B and C) which continued for several seconds after the applications. After such bursts of excitation the responsiveness of neurones remained depressed for some seconds.

Dopamine and 5-hydroxytryptamine also depressed the responses of cortical neurones to synaptic excitation. The two units illustrated in Fig. 3A were responding consistently to stimulation of the contralateral hindpaw. However, 16 sec after starting the application of dopamine, both units had ceased to discharge. Recovery after the end of the application was complete within a few seconds. Another unit is featured in Fig. 3C. Each record in this instance represents several superimposed responses to stimulation of the contralateral forepaw. After the controls, 5-hydroxytryptamine was applied (current 70 nA) and the evoked discharges disappeared. There was a rapid recovery when the application of 5-hydroxytryptamine ceased. The effect of lysergic acid diethylamide on synaptic transmission is also shown in Fig. 3B. This unit responded consistently with a single spike to stimulation of the contralateral forepaw. After 18 sec of passing a current of 80 nA through the barrel containing lysergic acid diethylamide, the unit failed to respond to the peripheral volley. In contrast to observations made with the lateral geniculate body (Curtis & Davis, 1962), we have found excitation by glutamate easier to block than synaptic responses, which usually required larger and longer applications of inhibitory drugs for a clear effect.

Characteristically lysergic acid diethylamide and the other lysergic acid derivatives depressed cortical neurones for very much longer than did catechol amines or simple indole derivatives. An example of this prolonged depression, as indicated by the responses to repeated applications of glutamate, is shown in Fig. 4. Ergometrine was the derivative of lysergic acid used in this instance, for it proved to be an even more active depressant than lysergic acid diethylamide. After 5 sec from the start of the release of ergometrine by a current of 40 nA, the responses to glutamate were

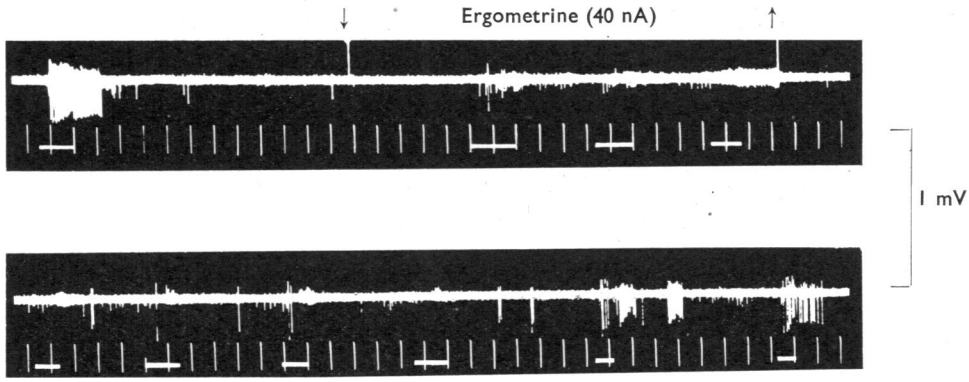

Fig. 4. Prolonged block by ergometrine of excitation due to L-glutamate. The cortical unit was initially excited by iontophoretic release of L-glutamate (60 nA) at the first white line below the upper trace, before 18 sec of release of ergometrine (between the two arrows). Later applications of glutamate (shown by subsequent white lines) were largely ineffective until about 30 sec after the end of the release of ergometrine. The cat was anaesthetized with allobarbitone. Time marks, 1 sec.

almost completely absent and two further applications of glutamate were ineffective. The cell remained in this inexcitable state for 30 sec after the end of the release of ergometrine, and even after 40 sec the response to glutamate had not fully recovered. Like some other compounds already mentioned, lysergic acid diethylamide and several derivatives also had excitant properties, which were sometimes revealed by application of larger amounts.

Compounds in these three groups were also tested on the antidromically evoked spikes of single Betz cells (Phillips, 1956, 1959 ; Krnjević & Phillis, 1963b). It has already been reported that, unlike the antidromic invasion of motoneurones in the spinal cord (Curtis *et al.*, 1959), the antidromic invasion of Betz cells is not readily blocked by γ-aminobutyric acid (Krnjević & Phillis, 1963a), although enormous doses of γ-aminobutyric acid may partially block invasion (Krnjević, 1963). The safety factor for antidromic invasion of Betz cells being considerably higher than that for motoneurones (Phillips, 1959), it was not surprising to find that dopamine, 5-hydroxytryptamine and lysergic acid diethylamide also failed to prevent antidromic excitation. The depression by catechol amines and 5-hydroxytryptamine of firing induced by glutamate was to some extent competitive, for in most instances a greater release of glutamate overcame the block. Attempts were also made with several cells to determine whether lysergic acid diethylamide, in amounts too small to depress directly the neurone, would prevent depression by 5-hydroxytryptamine ; it was never possible to demonstrate such an antagonism.

Section II

In this section the results with several groups of compounds will be discussed. Compounds in the first three groups were selected largely on the basis of their structural similarity to the compounds mentioned in Section I. Table 1 presents a number of compounds related to dopamine and adrenaline ; Table 2, various simple derivatives of 5-hydroxytryptamine ; Table 3, derivatives of lysergic acid ; and Table 4, some miscellaneous compounds including imidazole derivatives, two antagonists of the sympathomimetic amines, two substances which antagonize 5-hydroxytryptamine, and adenosine triphosphate.

The depressant potencies of compounds in the first three groups were compared primarily with the potency of the group representative discussed in Section I, but the more active compounds were also compared directly with dopamine. In each instance we determined the iontophoretic current required to produce a depression of the neuronal response similar to that caused by a given iontophoretic current through the barrel containing the group representative. For the purpose of this investigation, it was assumed that each of these compounds has a similar transport number under these conditions, and potencies were taken to be inversely proportional to the magnitude of the respective currents. Closely related substances such as dopamine, adrenaline, noradrenaline and isoprenaline are likely to have similar transport numbers in comparable solutions, and any outflows by electro-osmosis will probably not be very different. The same consideration is likely to be true when comparing 4-, 5- and 6-hydroxytryptamine. It is by no means certain, however, that the iontophoretic current is so simply related to the release of drug when

TABLE 1

DERIVATIVES OF PHENETHYLAMINE

Depressant actions were tested on neurones excited by L-glutamate; the intensities are expressed by comparing approximately equipotent iontophoretic currents, taking dopamine as the standard of reference indicated by ———. Excitatory effects of larger currents are indicated by (+), and apparently simple excitation by + or ++

Compound	Substitution in ring	Side-chain	Intensity of action	Time-course of action
DL-Phenylalanine		$CH_2.CH(NH_2).CO_2H$	——	
(±)-Amphetamine		$CH_2.CH(NH_2).CH_3$	— (+)	
(—)- and (+)-Ephedrine		$CH(OH).CH(NH.CH_3).CH_3$	———	
(—)- and (+)-Pseudoephedrine		$CH(OH).CH(NH.CH_3).CH_3$	———	Slow
N-Methylephedrine		$CH(OH).CH(CH_3).N(CH_3)_2$	———	
Norephedrine		$CH(OH).CH(NH_2).CH_3$	———	
Norpseudoephedrine		$CH(OH).CH(NH_2).CH_3$	———	
Tyramine	4-OH	$CH_2.CH_2.NH_2$	—	Quick
Tyrosine	4-OH	$CH_2.CH(NH_2).CO_2H$	?—	
DL- and L-Dopa	3-OH,4-OH	$CH_2.CH(NH_2).CO_2H$	+	Slow
Dopamine	3-OH,4-OH	$CH_2.CH_2.NH_2$	———	
Noradrenaline	3-OH,4-OH	$CH(OH).CH_2.NH_2$	—	
Adrenaline	3-OH,4-OH	$CH(OH).CH_2.NH.CH_3$	——(+)	Quick
Isoprenaline	3-OH,3-OH	$CH(OH).CH_2.NH.CH(CH_3)_2$	——	
Mescaline	$3\text{-}OCH_3,4\text{-}OCH_3 5\text{-}OCH_3$	$CH_2.CH_2.NH_2$	?—	

compounds of different types are being compared, such as imidazolylacetic acid and dopamine; but until more is known about quantitative aspects of the iontophoretic release of the various compounds, direct comparison of currents seems the most useful way of expressing relative potencies.

Dopamine was arbitrarily assessed as having a depressant potency of − − − and the other compounds were graded on this basis. Compounds, such as adrenaline and 5-hydroxytryptamine, which had a dual action, depressing at lower concentrations and exciting when larger amounts were applied, were tabulated as − −(+).

Derivatives of phenethylamine (Table 1)

Many of the phenethylamine derivatives were relatively potent, and the results can be summarized as follows:

(I) *Variations in the side-chain.* All the compounds in this group, with the exception of amphetamine, were fairly powerful depressants of cortical neurones. (−)-Ephedrine, the action of which is illustrated in Fig. 5, was the most active

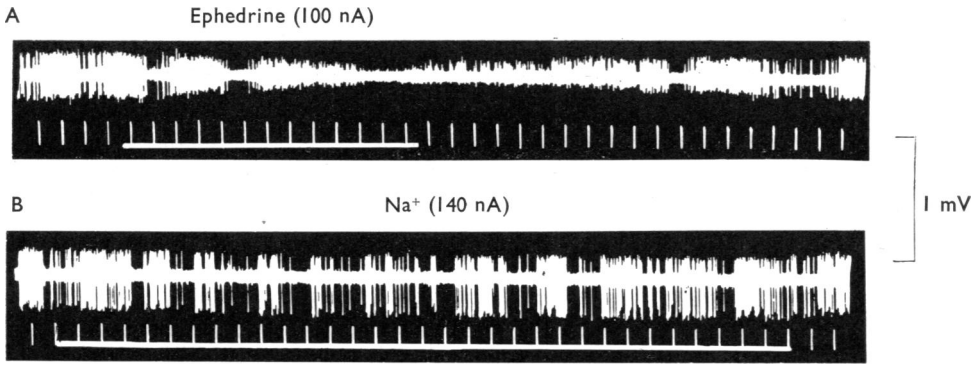

Fig. 5. A: block of spontaneous activity of a cortical unit by (−)-ephedrine, released iontophoretically during the time shown by the white line below trace. Note the slow recovery of spike amplitude. B: control record during release of Na^+ by an even larger current. The cat was anaesthetized with ether. Time marks, 1 sec.

compound tested in the series. The response shown in Fig. 5A was from a cortical unit firing rapidly and spontaneously; when a current of 100 nA was passed through the barrel containing ephedrine the spikes were progressively reduced in amplitude and frequency; recovery was slow, even though the application of ephedrine was comparatively short (14 sec), and the spontaneous firing took 25 to 30 sec to return to control frequency. The application of a large positive (outward) current releasing Na^+ from another barrel only slightly reduced the frequency of firing of the same unit and did not depress spike height (Fig. 5B).

Ephedrine is equally effective in blocking excitation caused by glutamate. This is shown in Fig. 6 where the action of ephedrine is compared directly with that of γ-aminobutyric acid. It is evident that even smaller releases of ephedrine can block the neuronal discharge, but this action has a slower onset and persists much longer

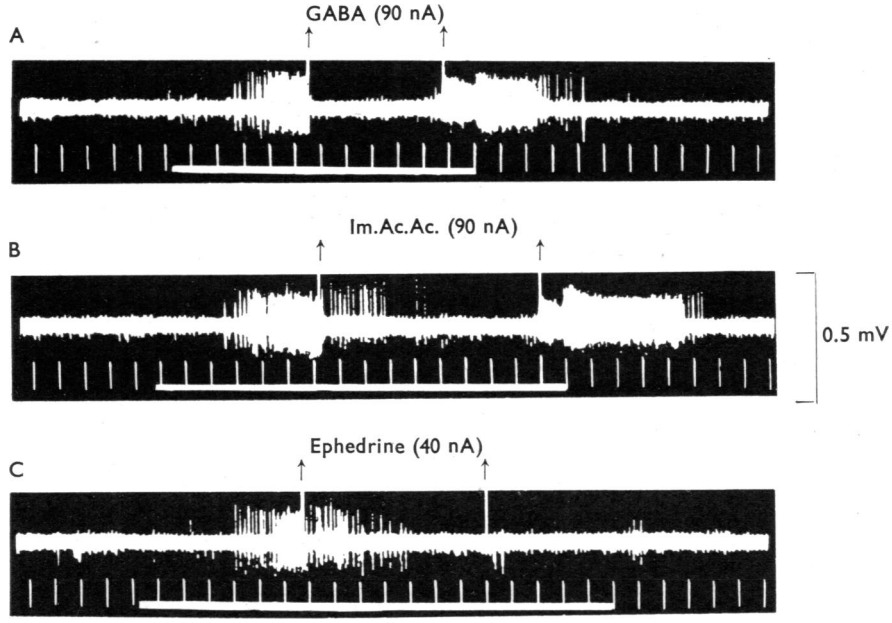

Fig. 6. Comparison of the blocking action of γ-aminobutyric acid (GABA, A), imidazolylacetic acid (Im.Ac.Ac., B) and (−)-ephedrine (C) on a cortical unit excited with L-glutamate, release of which is indicated by the white lines (current, 80 nA). Depressant agents were applied during periods between arrows. Note prolonged action of ephedrine. The cat was anaesthetized with allobarbitone. Time marks, 1 sec.

than that of γ-aminobutyric acid. Each of the four optical isomers of ephedrine produced a comparable effect. Three other derivatives of ephedrine were somewhat less active.

(II) *Monohydroxylation of the benzene ring.* Tyramine and tyrosine were tested as representatives of this group. Neither possessed more than very weak depressant properties.

(III) *Dihydroxylation of the benzene ring.* This group includes dopamine and adrenaline, as well as L- and DL-dihydroxyphenylalanine (L- and DL-dopa), noradrenaline and isoprenaline. The actions of dopamine and adrenaline have already been described ; isoprenaline was approximately equipotent with adrenaline, whilst noradrenaline was considerably weaker. It is of interest to note that Marrazzi (1957) also found that noradrenaline was much less potent than adrenaline. It has been reported recently that noradrenaline can excite or depress certain neurones in the brain stem (Bradley & Wolstencroft, 1962).

Dopa has been widely used as a substitute for the catechol amines in pharmacological investigations (Carlsson, Lindquist, Magnusson & Waldeck, 1958 ; Carlsson, 1959 ; Monnier, 1960 ; Mantegazzini & Glässer, 1960 ; Birkmayer & Hornyekiewicz, 1961), the blood–brain barrier in the cerebral cortex being relatively impermeable to the latter (Weil-Malherbe, Whitby & Axelrod, 1961) though permeable to the amino-acid dopa (Carlsson *et al.*, 1958 ; Bertler & Rosengren, 1959). In our experi-

ments, L- and DL-dopa excited cortical neurones in a manner somewhat like that seen with relatively large amounts of adrenaline and 5-hydroxytryptamine. The actions of dopamine and DL-dopa are compared in Fig. 7. Both excitation by glutamate and spontaneous firing were readily blocked by dopamine (Fig. 7A and C); in contrast, DL-dopa had no definite blocking action, but tended to cause either a slight and gradual increase in excitability (Fig. 7B) or, more often, a sudden paroxysmal discharge (Fig. 7D).

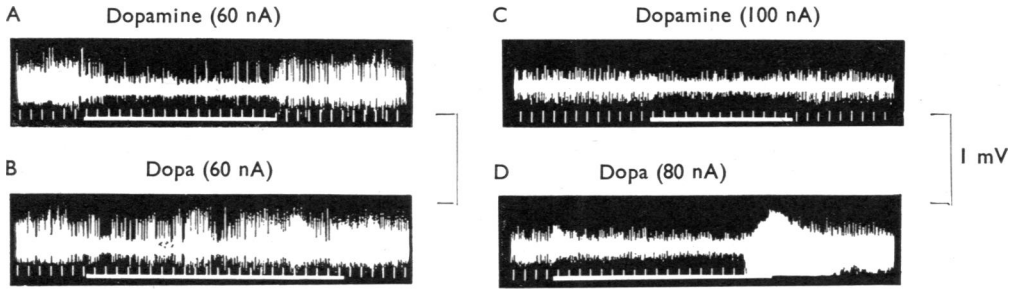

Fig. 7. Unlike dopamine, dopa tends to excite cortical neurones. In A and B, units were excited by a steady background release of L-glutamate (30 nA); in C and D, other units were firing spontaneously. Release of dopa and dopamine is indicated by the white lines. The cat was anaesthetized with allobarbitone. Time marks, 1 sec.

(IV) *Further substitution in the benzene ring.* The sole example of this group was mescaline, the psychotropic actions of which were first described by Heffter in 1896. According to Marrazzi & Hart (1955), mescaline weakly depresses the transcallosal cortical response and, when applied topically, it raises the threshold for direct cortical stimulation (Rovetta, 1956). In the present investigation mescaline was only rather feebly depressant.

Indole derivatives (Table 2). Section A, unsubstituted indole nucleus

(I) *Side-chain variation.* The tryptamine molecule represents the basic structure of the majority of compounds in Table 2, and its action was therefore of considerable interest. Though less potent than dopamine, it was at least as active in blocking the responses of cortical neurones as any of its analogues. Tryptophan, a precursor in the biosynthesis of 5-hydroxytryptamine which readily passes through the blood–brain barrier (Udenfriend, 1958), possessed a combination of excitant and depressant actions.

(II) *N-Alkylation of the tryptamine side-chain.* This change reduced the depressant activity.

Indole derivatives (Table 2). Section B, substitution in the indole nucleus

(I) *Position of the phenolic hydroxyl group.* The activities of 4-, 5 and 6-hydroxytryptamine were similar (Fig. 2) and similar in strength to that of tryptamine, whereas 7-hydroxytryptamine was somewhat less active. Carboxylation of the side-chain of 4-, 5- and 6-hydroxytryptamine to form the respective amino-acids resulted in a loss of activity.

TABLE 2

DERIVATIVES OF TRYPTAMINE

Depressant actions were tested on neurones excited by L-glutamate; the intensities are expressed by comparing approximately equipotent iontophoretic currents, taking dopamine as the standard of reference, indicated by ———. Excitatory effects of larger currents are indicated by (+) and apparently simple excitation by + or ++

Compound	Intensity of action	Time-course of action
Tryptamine	——	Quick
DL-Tryptophan	——(+)	Quick
Indol-3-ylacetic acid	0	
NN-Dimethyltryptamine	—	Quick
NN-Diethyltryptamine	—	Quick
4-Hydroxytryptamine	——	Quick
4-Hydroxytryptophan	—	Quick
5-Hydroxytryptamine	——(+)	Quick
5-Hydroxytryptophan	—	Quick
6-Hydroxytryptamine	——(+)	Quick
6-Hydroxytryptophan	—	Quick
7-Hydroxytryptamine	—(+)	Quick
4-Methoxytryptamine	—	Quick
5-Methoxytryptamine	—	Quick
4-Hydroxy-*NN*-dimethyltryptamine (psilocine)	—	Quick
5-Hydroxy-*NN*-dimethyltryptamine (bufotenine)	—	Quick
6-Hydroxy-*NN*-dimethyltryptamine	—	Quick
N-Acetyl-5-methoxytryptamine (melatonin)	0	

(II) *Methylation of the phenolic hydroxyl group.* This change reduced the depressant activity of 4- and 5-hydroxytryptamine.

(III) *N-Alkylation of the side-chain of 4- and 5-hydroxytryptamine.* This change yields psilocine and bufotenine each of which possesses hallucinogenic properties (Wasson, 1959; Fabing & Hawkins, 1956). However, these compounds, like the *N*-alkylation product of 6-hydroxytryptamine, were distinctly less active. Psilocine had no special excitatory properties (cf. Weidmann & Cerletti, 1960). Melatonin had no clear action.

Derivatives of lysergic acid (Table 3)

Lysergic acid derivatives were of interest because of their structural similarities to the simpler indole derivatives, their hallucinogenic properties and their antagonism of the actions of 5-hydroxytryptamine.

TABLE 3

DERIVATIVES OF LYSERGIC ACID

Depressant actions were tested on neurones excited by L-glutamate; the intensities are expressed by comparing approximately equipotent iontophoretic currents, taking dopamine as the standard of reference, indicated by ———. Excitatory effects of larger currents are indicated by (+) and apparently simple excitation by + or ++

Compound	Side-chain	Intensity of action	Time-course of action
D-Lysergic acid diethylamide	$CO.N(C_2H_5)_2$	——(+)	Slow
Ergometrine	$CO.NH.CH(CH_3).CH_2OH$	———(+)	Slow
Methylergometrine	$CO.NH.CH(C_2H_5).CH_2OH$	++	Slow
12-Hydroxyergometrine	$CO.NH.CH(CH_3).CH_2OH$	—(+)	Slow
Methysergide (UML-491)	$CO.NH.CH(C_2H_5).CH_2OH$	——(+)	Slow

(I) *Variations in the side-chain.* The actions of lysergic acid diethylamide and of ergometrine have already been described in Section I of this paper. It was mentioned there that lysergic acid diethylamide and its derivatives may cause excitation under some conditions. In the case of methylergometrine, this excitatory effect is predominant (Fig. 8). After the initial phase of excitation, the responses of the neurone to glutamate were depressed for about 2 min ; this was followed by several minutes when firing due to glutamate was greatly prolonged, indicating that the cell was still in a state of increased excitability. The period of depression immediately after the rapid discharge caused by methylergometrine probably resulted from a block by depolarization of the neurone.

(II) *Nucleus substitution.* The prolonged depression by methysergide of the firing due to glutamate is illustrated in Fig. 9. Larger amounts of methysergide caused

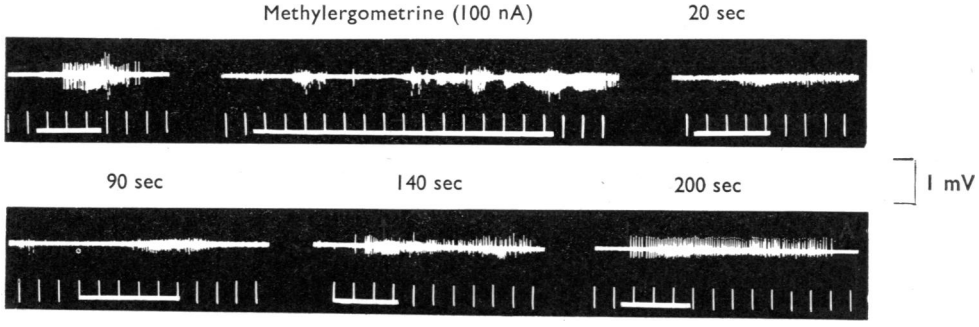

Fig. 8. Action of methylergometrine applied for 15 sec to a cortical unit, as shown by the white line in the second record of the upper row. The excitability of the unit was tested by several applications of L-glutamate (white lines in other records), one before and four after releasing methylergometrine, at the various times indicated. The cat was anaesthetized with allobarbitone. Note the excitation by methylergometrine and the prolonged change in response to glutamate. Time marks, 1 sec.

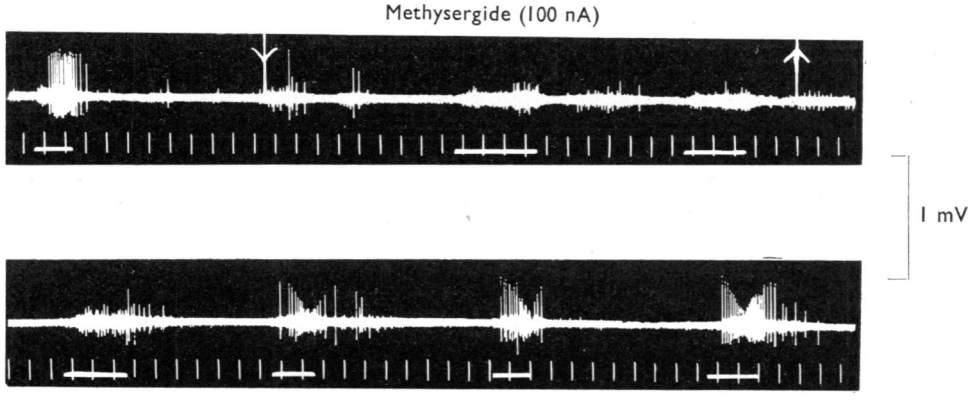

Fig. 9. Block by methysergide (applied between arrows) of excitation due to L-glutamate of a cortical unit. The horizontal white lines below the traces indicate release of L-glutamate (80 nA). The traces are continuous. The cat was anaesthetized with allobarbitone. Time marks, 1 sec.

a high frequency discharge of some neurones. 12-Hydroxyergometrine possessed weak excitant and depressant properties.

Miscellaneous compounds (Table 4)

(I) *Imidazole derivatives.* Histamine and the related imidazole derivatives were tested because they occur in the brain (Kwiatkowski, 1943; Adam, 1961) where

TABLE 4

MISCELLANEOUS COMPOUNDS

Depressant actions were tested on neurones excited by L-glutamate; the intensities are expressed by comparing approximately equipotent iontophoretic currents, taking dopamine as the standard of reference, indicated by ———. Excitatory effects of larger currents are indicated by (+) and apparently simple excitation by + or ++

Compound	Intensity of action	Time-course of action
	——	
Imidazolylacetic acid	——(+)	Quick
L-Histidine	——	Slow
Histamine	—	Quick
Chlorpromazine	0	
Dichloroisoprenaline	—(+)	Slow
Yohimbine	?—	
Harmine	0	
Adenosine triphosphate	0	

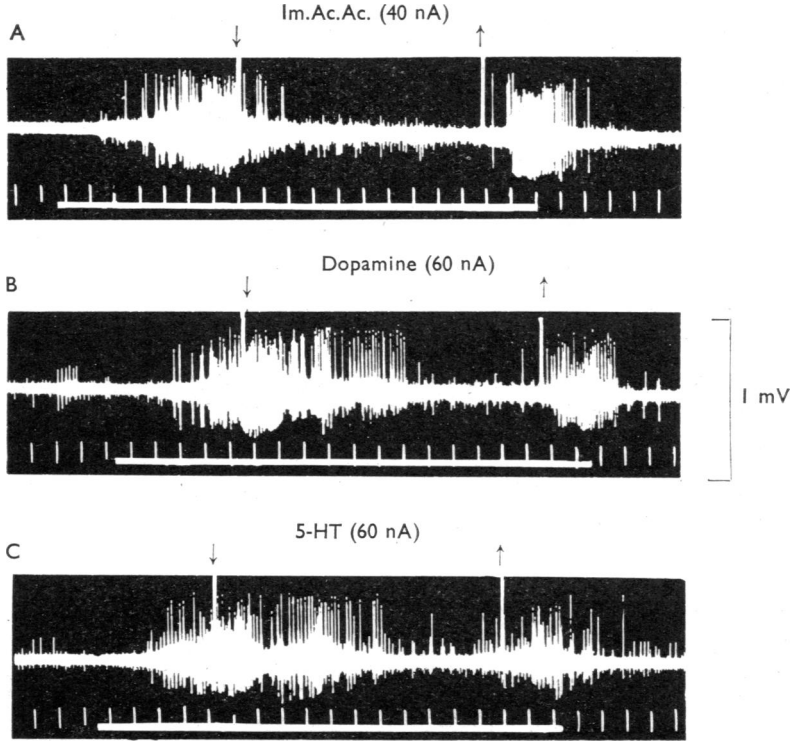

Fig. 10. Comparison of the effects produced by imidazolylacetic acid (Im.Ac.Ac., A), dopamine (B) and 5-hydroxytryptamine (5-HT, C) applied (between two arrows) to the same unit in the cerebral cortex of a cat anaesthetized with allobarbitone. In each case, the unit was excited by a similar release of L-glutamate (30 nA) signalled by white lines. Time marks, 1 sec.

they are actively metabolized (White, 1960). Although histamine and histidine were only weakly depressant, imidazolylacetic acid was highly active. Its depressant action on firing due to glutamate is illustrated in Figs. 6 and 10. The responses of the cells shown in both pictures were entirely suppressed. Fig. 10 demonstrates for comparison the blocking actions of dopamine and of 5-hydroxytryptamine applied from the same electrode and by greater currents. It is apparent that imidazolylacetic acid is more potent than dopamine and, as shown in Fig. 6, its blocking action is similar in strength to that of γ-aminobutyric acid, though it is somewhat slower in onset.

(II) *Antagonists of sympathomimetic amines.* Chlorpromazine and dichloroisoprenaline were tested primarily to see whether they modified the effects of dopamine and adrenaline. Though neither compound had any action in this regard, dichloroisoprenaline did cause some depression of excitation due to glutamate.

(III) *Yohimbine and harmine.* These compounds, which each contain an indole nucleus, are antagonists of 5-hydroxytryptamine (Shaw & Woolley, 1953) and may cause psychic disturbances (Iberico, 1941 ; Woolley & Shaw, 1954). Each was practically without effect on cortical neurones.

(IV) *Adenosine triphosphate.* This compound has been postulated as a neurotransmitter (Crossland, 1957) ; it was tested repeatedly, on a total of fifty-three cells in the cerebral cortex and fourteen cells in the cerebellar cortex, but it could not be shown to have any clear action, either excitatory or inhibitory.

DISCUSSION

The present studies were undertaken to investigate the actions on cortical neurones of various indole and catechol amines and related compounds. Considerable interest has been expressed in a possible role of some of these compounds as neurotransmitters, and others, which possess marked psychotropic properties, have frequently been thought to act by selectively modifying neuronal activity in the brain.

The majority of compounds tested depressed to some extent various kinds of neuronal discharge, including spontaneous firing, responses evoked by afferent nervous stimulation and excitation produced by the local application of glutamate. The finding that firing due to glutamate was depressed suggests that these drugs act directly on the postsynaptic membrane. The depression of synaptic transmission was therefore probably a consequence of this action, though the possibility of a presynaptic effect cannot be excluded entirely.

It is clear that, as far as depressant actions are concerned, the compounds studied here fall into two broad classes. In the first are the catechol amines, the simple indole derivatives and some imidazole compounds. Their action is relatively quick in onset and rapidly reversible. The principal effect on neuronal firing is a marked reduction in frequency, and there is little depression of spike amplitude.

The second class consists of lysergic acid diethylamide and some of its derivatives, and of certain phenethylamines such as ephedrine. These drugs depress the

amplitude of neuronal spikes markedly, the effect being relatively slow in onset and only slowly reversible. There is a much smaller change in the rate of discharge before the onset of complete block.

The depressant action of compounds in the first class is somewhat like that of the short-chain ω-amino-monocarboxylic acids, such as γ-aminobutyric acid (Krnjević & Phillis, 1961, 1963a), which are believed to act mainly by increasing the permeability of the cell membrane (Curtis *et al.*, 1959 ; Curtis & Watkins, 1960). The catechol amines probably trigger their effects as a result of an electrostatic interaction with receptor sites (Belleau, 1960). They may perhaps be considered as comparable to an ω-amino-acid, with a positive and negative charge at least potentially available at each end of the molecule, although the phenolic hydroxyl groups are in fact likely to be only slightly ionized at a body *p*H (Lewis, 1954). This analogy is given some support by the contrasting properties of dopa and dopamine. Dopa only excited cells, in this respect being like the excitatory dicarboxylic amino-acids which yield depressant compounds after α-decarboxylation (Curtis & Watkins, 1960 ; Krnjević & Phillis, 1961, 1963a). However, only a limited parallel can be drawn between these amines and the amino-acids: thus 5-hydroxytryptophan, the acid precursor of 5-hydroxytryptamine, did not tend to excite cortical cells.

The excitatory actions of relatively large amounts of compounds like 5-hydroxy-tryptamine and adrenaline resemble those of long-chain ω-amino-monocarboxylic acids, which also possess both depressant and excitant properties (Hayashi, 1956 ; Krnjević & Phillis, 1963a). It is possible that compounds which do not combine readily with the postulated receptor responsible for depression of activity may react with the membrane in an alternative manner leading to excitation. On the other hand, it is also quite likely that the presence of sufficient amounts of compounds with a strong positive charge may have a non-specific, disruptive effect on the cell membrane, causing a temporary breakdown of its electrical resistance, and thereby the observed discharge [compare the actions of nicotine, hydrogen ions, etc. (Krnjević & Phillis, 1963a)].

The effects produced by depressant compounds in the second class clearly resemble those associated with substances having local anaesthetic properties, such as procaine and atropine (Curtis & Phillis, 1960 ; Krnjević & Phillis, 1963a). There is good evidence that such compounds interfere with the selective changes in membrane permeability which are essential for the generation of the spike during activity (Shanes, 1958). It is therefore significant that the main compounds in this group, lysergic acid diethylamide and ephedrine, are each quite effective as local anaesthetics (Di Carlo, 1961 ; Schultz, 1940). Some of these compounds, such as lysergic acid diethylamide and its various derivatives, also have excitatory properties, usually revealed by the application of a larger amount. This effect was seen particularly clearly with methylergometrine, whose " depressant " action apparently resulted from a very powerful and prolonged excitation which probably kept the neurones blocked by an excess of depolarization. Such effects are not always readily distinguished from other types of depression, for example that due to hyperpolarization.

The physiological significance of these results is uncertain. The concentrations of the drugs in the region of the neurones under observation were probably not

less than 10^{-4} M (Krnjević & Phillis, 1963a). Such relatively large concentrations may perhaps be associated with non-specific effects, differing in kind from those responsible for psychotropic phenomena. On the other hand, we studied only pronounced changes in neuronal responsiveness. Marginal blocking effects may be produced by very much smaller doses, especially if a cumulative action can develop slowly ; such effects would block many synapses with a low safety factor for transmission, thus probably changing significantly the behaviour of large populations of cells. It is of some relevance that Marrazzi was able to demonstrate a similar action of many of these compounds on transcallosally evoked cortical responses (Marrazzi & Hart, 1955 ; Marrazzi, 1957) while using relatively low doses of the drugs ; for example, 1 μg/kg of 5-hydroxytryptamine, injected into the carotid artery, was sufficient to decrease substantially the magnitude of the response. Criticism of these experiments by Koella, Smythies, Bull & Levy (1960) and Koella (1960) appears to have been satisfactorily answered by further experiments (Rodriguez, Hart & Marrazzi, 1961 ; Hart, Rodriguez & Marrazzi, 1961). Furthermore Purpura has studied the actions of intravenously injected lysergic acid diethylamide on responses in the auditory and the visual cortex (Purpura, 1956a and b). Although the effects were complex, depending on the dose, he was able to demonstrate an inhibition of auditory responses with doses of 40 to 60 μg/kg. Grenell (1957) found that intravenous doses (10 μg/kg) of lysergic acid diethylamide were sufficient to depress the cortical responses to direct stimulation. Although these doses are considerably higher than those required to produce mental aberrations in man, it must be borne in mind that a considerable disturbance of cerebral function would be associated with such gross changes in the evoked potentials.

There have been several suggestions that dopamine and 5-hydroxytryptamine in the brain may subserve inhibitory functions. Dopamine occurs in large quantities in the corpus striatum and may be concerned with the control of the extra-pyramidal cortico-spinal mechanisms, for depletion of the brain dopamine by administration of reserpine causes a Parkinson-type of syndrome which can be relieved by the administration of the precursor of dopamine, dopa (Carlsson, 1959). Moreover, it appears that a substantial proportion of the dopamine found in the caudate nucleus is bound in a tissue fraction rich in nerve endings (R. Laverty, I. A. Michaelson, D. F. Sharman & V. P. Whittaker, personal communication). It has also been suggested that the improvement which is seen in epileptic patients given iproniazid, an inhibitor of monoamine-oxidase, is due to increased levels of brain 5-hydroxytryptamine (Santanelli, Municchi & Serra, 1961).

The present conclusions about the actions of various amines are in marked contrast to those reached by Curtis & Davis (1962) after a similar study on the pharmacology of the lateral geniculate nucleus. In this latter study, not only were the derivatives of phenethylamine almost entirely devoid of activity but in addition the simple derivatives of indole and lysergic acid blocked synaptic transmission without affecting firing due to glutamate. Hence the authors postulated that the release or action of the transmitter was being interfered with, and suggested that the transmitter may be structurally related to 5-hydroxytryptamine and to lysergic acid diethylamide.

NOTE ADDED IN PROOF

A quantitative study of the iontophoretic release of some amines from micropipettes (see following paper) has shown substantial variations in transport number in different pipettes filled with the same solution. Nevertheless it appears that 5-hydroxytryptamine consistently has a relatively low transport number when released from solutions of 5-hydroxytryptamine creatinine sulphate. This substance and some of its derivatives may therefore have a somewhat greater potency than was assigned to them in Table 2.

REFERENCES

ADAM, H. M. (1961). Histamine in the central nervous system and hypophysis of the dog. In *Regional Neurochemistry. The Reginal Chemistry, Physiology and Pharmacology of the Nervous System*, ed. KETY, S. S. & ELKES, J., pp. 293–304. London: Pergamon.

ALBERT, A. (1952). Ionization, *p*H and biological activity. *Pharmacol. Rev.*, **4**, 136–167.

AMIN, A. H., CRAWFORD, T. B. B. & GADDUM, J. H. (1954). Active substances in brain extracts. *J. Physiol. (Lond.)*, **126**, 596–618.

BELLEAU, B. (1960). Relationships between agonists, antagonists and receptor sites. In *Adrenergic Mechanisms*, ed. VANE, J. R., WOLSTENHOLME, G. E. W. & O'CONNOR, M., pp. 223–245. London: Churchill.

BERTLER, A. & ROSENGREN, E. (1959). Occurrence and distribution of catechol amines in brain. *Acta physiol. scand.*, **47**, 350–361.

BIRKMAYER, W. & HORNYEKIEWICZ, O. (1961). Der L-3, 4-Dioxyphenylanin(=Dopa)-Effekt bei der Parkinson-Akinese. *Wien. klin. Wschr.*, **45**, 787–788.

BOGDANSKI, D. F., WEISSBACH, H. & UDENFRIEND, S. (1957). The distribution of serotonin, 5-hydroxytryptophan decarboxylase and monoamine oxidase in brain. *J. Neurochem.*, **1**, 272–278.

BRADLEY, P. B. & WOLSTENCROFT, J. H. (1962). Excitation and inhibition of brain-stem neurones by nor-adrenaline and acetylcholine. *Nature (Lond.)*, **196**, 840, 873.

CARLSSON, A. (1959). The occurrence, distribution and physiological role of catechol amines in the nervous system. *Pharmacol. Rev.*, **11**, 490–493.

CARLSSON, A., LINDQUIST, M., MAGNUSSON, T. & WALDECK, B. (1958). On the presence of 3-hydroxytyramine in brain. *Science*, **127**, 471.

CROSSLAND, J. (1957). The problem of non-cholinergic transmission in the central nervous system. In *Metabolism of the Nervous System*, ed. RICHTER, D., pp. 523–541. London: Pergamon.

CURTIS, D. R. (1962). Action of 3-hydroxytyramine and some tryptamine derivatives on spinal neurones. *Nature (Lond.)*, **194**, 292.

CURTIS, D. R. & DAVIS, R. (1962). Pharmacological studies upon neurones of the lateral geniculate nucleus of the cat. *Brit. J. Pharmacol.*, **18**, 217–246.

CURTIS, D. R. & ECCLES, R. M. (1958). The excitation of Renshaw cells by pharmacological agents applied electrophoretically. *J. Physiol. (Lond.)*, **141**, 435–445.

CURTIS, D. R. & KOIZUMI, K. (1961). Chemical transmitter substances in the brain stem of the cat. *J. Neurophysiol.*, **24**, 80–90.

CURTIS, D. R. & PHILLIS, J. W. (1960). The action of procaine and atropine on spinal neurones. *J. Physiol. (Lond.)*, **153**, 17–34.

CURTIS, D. R., PHILLIS, J. W. & WATKINS, J. C. (1959). The depression of spinal neurones by γ-amino-n-butyric acid and β-alanine. *J. Physiol. (Lond.)*, **146**, 185–203.

CURTIS, D. R., PHILLIS, J. W. & WATKINS, J. C. (1960). The chemical excitation of spinal neurones by certain acidic amino acids. *J. Physiol. (Lond.)*, **150**, 656–682.

CURTIS, D. R. & WATKINS, J. C. (1960). The excitation and depression of spinal neurones by structurally related amino acids. *J. Neurochem.*, **6**, 117–141.

DI CARLO, V. (1961). Ricerche sul meccanismo di azione degli anestetici locali. *Arch. ital. Sci. farmacol.*, **11**, 3–12.

FABING, H. D. & HAWKINS, J. R. (1956). Intravenous bufotenine injection in the human. *Science*, **123**, 886–887.

GRENELL, R. G. (1957). Mechanisms of action of psychotherapeutic and related drugs. *Ann. N.Y. Acad. Sci.*, **66**, 826–835.

HART, E. R., RODRIGUEZ, J. M. & MARRAZZI, A. S. (1961). Carotid and vagal afferents and drug action on transcallosally evoked cortical potentials. *Science*, **134**, 1696–1697.

HAYASHI, T. (1956). *Chemical Physiology of Excitation in Muscle and Nerve*, p. 159. Tokyo: Nakayama-Shoten.

HEFFTER, A. (1896). Über Cacteenalkaloide. *Ber. dtsch. chem. Ges.*, **29**, 216–227.

HILL, R. B. & USHERWOOD, P. N. R. (1961). The action of 5-hydroxytryptamine and related compounds on neuromuscular transmission in the locust *Schistocerca gregaria*. *J. Physiol. (Lond.)*, **157**, 393–401.

IBERICO, C. C. (1941). Ayahuasca. *Bol. Mus. Hist. Nat. (Lima)*, **5**, 313. Cited by EVARTS, E. V. (1957). A review of the neurophysiological effects of lysergic acid diethylamide (LSD) and other psychomimetic agents. *Ann. N.Y. Acad. Sci.*, **66**, 479–495.

KOELLA, W. P. (1960). Physiological fractionation of the effect of serotonin on evoked potentials. In J. Macy Foundation-Transactions, *Neuropharmacology, V*, ed. ABRAMSON, H. A., pp. 199–223. New Jersey: Madison Printing Co.

KOELLA, W. P., SMYTHIES, J. R., BULL, D. M. & LEVY, C. K. (1960). Physiological fractionation of the effect of serotonin on evoked potentials. *Amer. J. Physiol.*, **198**, 205–212.

KRNJEVIĆ, K. (1963). Iontophoretic studies with micropipettes. In *Neuro-psychopharmacology*, vol. 3, ed. BRADLEY, P. B., *et al.* Proceedings of 3rd Meeting of Coll. Int. Neuropsychopharmacol. Amsterdam: Elsevier, in the press.

KRNJEVIĆ, K. & MITCHELL, J. F. (1961). A simple and reliable device utilising transistors for the maintenance of a constant body temperature. *J. Physiol. (Lond.)*, **158**, 6–8*P*.

KRNJEVIĆ, K. & PHILLIS, J. W. (1961). The actions of the certain amino acids on cortical neurones. *J. Physiol. (Lond.)*, **159**, 62–63*P*.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963a). Iontophoretic studies of neurones in the mammalian cerebral cortex. *J. Physiol. (Lond.)*, **165**, 274–304.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963b). Acetylcholine sensitive cells in the cerebral cortex. *J. Physiol. (Lond.)*, in the press.

KWIATKOWSKI, H. (1943). Histamine in nervous tissue. *J. Physiol. (Lond.)*, **102**, 32–41.

LEWIS, G. P. (1954). The importance of ionization in the activity of sympathomimetic amines. *Brit. J. Pharmacol.*, **9**, 488–493.

MALCOLM, J. (1958). Effects of 5-hydroxytryptamine on evoked responses in the brain of anaesthetized cats. In *5-Hydroxytryptamine*, ed. LEWIS, G. P., pp. 221–228. London: Pergamon.

MANTEGAZZINI, P. & GLÄSSER, A. (1960). Action de la DL-3,4 dioxyphenylalanine (DOPA) et de la dopamine sur l'activité électrique du chat " cerveau isolé." *Arch. ital. Biol.*, **98**, 357–374.

MARRAZZI, A. S. (1957). The effects of certain drugs on cerebral synapses. *Ann. N.Y. Acad. Sci.*, **66**, 496–507.

MARRAZZI, A. S. & HART, E. R. (1955). Evoked cortical responses under the influence of hallucinogens and related drugs. *Electroenceph. clin. Neurophysiol.*, **7**, 146.

MONNIER, M. (1960). Actions électro-physiologiques des stimulants due système nerveux central. I. Systèmes adrénergiques, cholinergiques et neurohumeurs sérotoniques. *Arch. int. Pharmacodyn.*, **124**, 281–301.

OCHS, S., BOOKER, H. & APRISON, M. H. (1960). Serotonin on direct cortical responses. *Physiologist*, **3**, 121.

PHILLIPS, C. G. (1956). Intracellular records from Betz cells in the cat. *Quart. J. exp. Physiol.*, **41**, 58–69.

PHILLIPS, C. G. (1959). Actions of antidromic pyramidal volleys on single Betz cells in the cat. *Quart. J. exp. Physiol.*, **44**, 1–25.

PHILLIS, J. W. & KRNJEVIĆ, K. (1962). The actions of certain amino acids and amines on single neurones of the cerebral cortex. *Abst. 22nd Int. Physiol. Congr.*, No. 837.

PURPURA, D. P. (1956a). Electrophysiological analysis of psychotogenic drug action. I. Effect of LSD on specific afferent systems in the cat. *Arch. Neurol. Psychiat. (Chic.)*, **75**, 122–131.

PURPURA, D. P. (1956b). Electrophysiological analysis of psychotogenic drug action. II. General nature of lysergic acid diethylamide (LSD) action on central synapses. *Arch. Neurol. Psychiat. (Chic.)*, **75**, 132–143.

RODRIGUEZ, J. M., HART, E. R. & MARRAZZI, A. S. (1961). Synaptic inhibition by serotonin in isolated cerebral cortex. *Pharmacologist*, **3**, 68.

ROTHBALLER, A. B. (1959). The effects of catecholamines on the central nervous system. *Pharmacol. Rev.*, **11**, 494–547.

ROVETTA, P. (1956). Effect of mescaline and LSD on evoked responses especially of the optic system of the cat. *Electroenceph. clin. Neurophysiol.*, **8**, 15–24.

SANTANELLI, R., MUNICCHI, L. & SERRA, C. E. (1961). EEG changes in epileptic patients induced by iproniazide treatment. *Electroenceph. clin. Neurophysiol.*, **13**, 136.

SCHULTZ, F. H. (1940). The local anesthetic properties of ephedrine hydrochloride. *Anesthesiology*, **1**, 69–71.

SHANES, A. M. (1958). Electrochemical aspects of physiological and pharmacological action in excitable cells. *Pharmacol. Rev.*, **10**, 165–273.

SHAW, E. & WOOLLEY, D. W. (1953). Yohimbine and ergot alkaloids as naturally occurring anti-metabolites of serotonin. *J. biol. Chem.*, **203**, 979–989.

STOLL, W. A. (1947). Lysergsäure—diäthylamid, ein Phantastikum aus der Mutterkorngruppe. *Schweiz. Arch. Neurol. Psychiat.*, **60**, 279–323.

TUCKERMAN, M. M., MAYER, J. R. & NACHOD, F. (1959). Anomalous pKa values of some substituted phenylethylamines. *J. Amer. chem. Soc.*, **81**, 92–94.

UDENFRIEND, S. (1958). Serotonin metabolism and the central nervous system. In *Biochemistry of the Central Nervous System*, ed. BRÜCKE, F., pp. 301–309. London: Pergamon.

VANE, J. R. (1959). The relative activities of some tryptamine analogues on the isolated rat stomach strip preparation. *Brit. J. Pharmacol.*, **14**, 87–98.

VOGT, M. (1954). The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. *J. Physiol. (Lond.)*, **123**, 451–481.

WASSON, R. G. (1959). The hallucinogenic mushrooms of Mexico: an adventure in ethnomycological exploration. *Trans. N.Y. Acad. Sci.*, **21**, 325–339.

WEIDMANN, H. & CERLETTI, A. (1960). Studies on Psylocibin and related compounds. *Helv. physiol. pharmacol. Acta*, **18**, 174–182.

WEIL-MALHERBE, H., WHITBY, L. G. & AXELROD, J. (1961). The blood-brain barrier for catecholamines in different regions of the brain. In *Regional Neurochemistry: the Regional Chemistry, Physiology and Pharmacology of the Nervous System*, ed. KETY, S. S. & ELKES, J., pp. 284–291. London: Pergamon.

WHITE, T. (1960). Formation and catabolism of histamine in cat brain *in vivo*. *J. Physiol. (Lond.)*, **152**, 299–308.

WOOLLEY, D. W. & SHAW, E. (1954). A biochemical and pharmacological suggestion about certain mental disorders. *Proc. nat. Acad. Sci. (Wash.)*, **40**, 228–231.