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CENTRAL EFFECTS PRODUCED BY INJECTION OF 48/80 INTO THE CEREBRAL VENTRICLES OF MICE

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Injections of 20 to 30 μg. of 48/80 into the cerebral ventricular spaces of mice produced a condition of motor excitement termed "delirium ambulatorium." This condition was aggravated by previous intraperitoneal injection of tranquillizers, atropine, and promethazine, not affected by previous injection of mepyramine and abolished by a previous injection of pentobarbitone. When an intraperitoneal injection of strychnine was followed by an intraventricular injection of 48/80, the strychnine convulsions became more violent, but the pattern of delirium ambulatorium was not produced.

Compound 48/80 has powerful histamine releasing properties (Paton, 1951; Feldberg and Paton, 1951; Feldberg and Talesnik, 1953), and under certain conditions also releases 5-hydroxytryptamine from mast cells. These disintegrate under the influence of 48/80 (Bhattacharya and Lewis, 1956; Parratt and West, 1957). However, its high toxicity and lethal effects cannot be attributed entirely to the systemic effects of released histamine or 5-hydroxytryptamine since the signs elicited by giving 48/80 to guinea-pigs are not typical of histamine poisoning (Mota and Vugman, 1956; Mota, 1957), and the amounts of 5-hydroxytryptamine released are too small to produce lethal effects in most species. Some of the toxic effects of 48/80 in guinea-pigs and rats may result from stimulation of the central nervous system. This suggestion is borne out by the following experiments in which 48/80 was injected intracerebrally of which a preliminary report was made at the tenth annual meeting of the Brazilian Society for the Advancement of Science in São Paulo, July 6 to 12, 1958.

METHODS

The experiments were performed on mice weighing between 18 and 22 g. Mice were chosen because they are relatively insensitive to the systemic effects of histamine. The 48/80 was injected in saline solution into the cerebral ventricles through the intact skull 2 to 3 mm. in front of the line joining the anterior base of the ears using a fine 26 gauge needle 0.5 cm. long according to the method described by Haley (1957) and Haley and McCormick (1957). The volume of injection was between 0.02 and 0.09 ml.

In order to find out if the effects of intracerebral 48/80 were influenced by other drugs, these were injected intraperitoneally in a volume of 0.1 to 0.2 ml. of saline solution some time before the intracerebral injection of 48/80. The time interval between the two injections was between 20 and 45 min., except with reserpine which was given 2.5 hr. before 48/80. Perphenazine (Trilafon: 1(2 - hydroxyethyl) - 4,3(2 - chlor-10 phenylthiazyl-propyl) piperazine) was also used.

RESULTS

Within a few minutes an intracerebral injection of 20 to 30 μg. of 48/80 led to a condition of deep depression. This lasted for 2 to 3 min., then the mouse suddenly started to run forward with well-co-ordinated but extremely quick movements. It either moved in a straight line or in wide circles. Convulsions were rarely seen at this early stage. The mouse seemed to have lost all sense of boundaries. When it jumped from a table it continued its forward course as soon as it reached the floor, until it hit an obstacle, and there were often periods in which the mouse continued to roll over on its side for many seconds. This "psychotic" behaviour has been termed "delirium ambulatorium." It ended usually after 5 to 10 min. by death or exhaustion or by a prolonged hyperkinetic state of jerky movements or of clonic convulsions. 80% of the injected animals developed all the signs of the delirium ambulatorium and half of these remained in a hyperkinetic state for many hours. In two mice signs of delirium ambulatorium with periods of rolling over continued for 24 hr. Occasionally animals died with symptoms of respiratory arrest

after 5 to 10 min. of intense running activity; this happened more frequently with higher doses (40 μg.) of 48/80.

Effects of Drugs on the Delirium Ambulatorium and Mortality Produced by 48/80

The results are summarized in Table I. Each drug was tested in groups of five mice to find out if it influenced the delirium ambulatorium and the mortality which was about 10% following the injection of 20 to 30 μg. of 48/80 alone.

When 48/80 was injected in mice during pentobarbitone anaesthesia, it greatly prolonged the sleeping time due to the barbiturate. The only stimulating effects produced by the injection of 48/80 in the anaesthetized animals were an increase in depth and rate of respiration and an increased general reflex excitability, particularly to touch and noise.

When given together with either promethazine or chlorpromazine before the injection of 48/80, pentobarbitone prevented not only the occurrence of the delirium ambulatorium but also the high mortality of mice treated with either promethazine or chlorpromazine before the administration of 48/80.

Table I shows that the two antihistamine drugs tested, promethazine and mepyramine, influenced the effects of the intracerebral injection of 48/80 in different ways. Promethazine strongly enhanced the delirium ambulatorium and increased the mortality to 60% whereas mepyramine influenced neither the delirium ambulatorium nor the mortality. This difference is probably not due to the fact that promethazine has a more potent antihistaminic effect than mepyramine. The difference may well be due to the fact that promethazine has also atropine-like properties, since atropine also increased the delirium ambulatorium and the mortality caused by 48/80 injections. The enhancing effect of perphenazine, another phenothiazine derivative, may also result from its atropine-like properties.

TABLE I

EFFECTS OF DRUGS INJECTED INTRAPERITONEALLY ON THE DELIRIUM AMBULATORIUM AND MORTALITY IN MICE PRODUCED BY SUBSEQUENT INTRACEREBRAL INJECTIONS OF 20 TO 30 μG. OF 48/80

All doses in mg./kg. body weight except reserpine and strychnine (mg./animal).

Drug Injected Before 48/80		Effect on Delirium Ambulatorium	% Mortality
Reserpine	2	Slight increase	0
Serpasol	5	Large ,,	80
Chlorpromazine	12	Slight ,,	40
	25	,, ,,	20
Atropine sulphate	1·25	Large ,,	60
Mepyramine maleate	50	No effect	0
Perphenazine	5	Large increase	100
Strychnine	0·02	Tonic convulsions	60
Promethazine hydrochloride	12·5	Large increase	80
Pentobarbitone	30–50	Abolition	0
,, plus			
(a) Promethazine	12·5	,,	0
(b) Chlorpromazine	12	,,	0
(c) Mepyramine	50	,,	0

In the experiments with strychnine, a dose was chosen which was not lethal and which produced only mild convulsions. The subsequent injection of 48/80 did not alter the pattern of the strychnine convulsions; they became violent but the condition did not revert to the pattern of delirium ambulatorium.

It is interesting to note that the powerful tranquillizers, chlorpromazine, promethazine, and perphenazine, increase the delirium ambulatorium and lethality of the 48/80 injections and that reserpine and Serpasol (Ciba) have a similar effect in doses which, when given alone, would produce a tranquillizing effect.

Discussion

The peculiar motor excitement produced in mice by intracerebral injections of 48/80 and termed delirium ambulatorium is probably due to stimulation of subcortical structures lining the ventricular spaces. It is, at present, not possible to state whether the effect is produced indirectly by the release of histamine, 5-hydroxytryptamine or some unknown substance, or whether it is the result of a direct stimulating action of 48/80 itself. It is interesting and relevant that 48/80 is not the only histamine liberator which on intracerebral injection produces the peculiar motor excitement; tubocurarine, which is also a histamine liberator, produces on injection into the cerebral ventricles of cats and mice a pattern of motor excitement which resembles in many features the delirium ambulatorium produced by 48/80 (Feldberg and Sherwood, 1954; Haley, 1957).

The fact that intracerebral injections of histamine or 5-hydroxytryptamine, even in large doses, do not produce in mice motor effects resembling delirium ambulatorium (Haley, 1957; Rocha e Silva, unpublished observations) does not exclude the possibility that these substances have such an action when released close to the reacting structures in the brain. If the central effect of 48/80 were due to release of histamine, it could not be caused by an action of histamine on the cerebral vessels because the effect persisted and was even increased after the injection of antihistaminic drugs which are known to reduce

greatly the vascular effects of released histamine (Virno, Gertner, and Bovet, 1956). On the other hand, since histamine releases catechol amines from the suprarenals, an analogous effect on these substances in the brain may be another way in which released histamine could produce central effects.

If the central effects of 48/80 result from the release of 5-hydroxytryptamine or catechol amines, the influence of reserpine which somewhat increases the delirium ambulatorium due to 48/80 is of interest, although this effect can be interpreted against as well as in favour of such an indirect mode of action of 48/80. Reserpine does not produce delirium ambulatorium although it reduces the 5-hydroxytryptamine and catechol amine content of the brain. This might seem to oppose the idea that these amines are involved in the production of the delirium ambulatorium. However, the fact that reserpine requires several hours for its action, whereas the effect of 48/80 occurs within a few minutes, might account for the difference, and the augmentation by reserpine of the delirium ambulatorium produced by 48/80 might be interpreted as a summation of a chronic with an acute release of these amines.

The finding that intracerebral 48/80 prolongs the sleeping time of pentobarbitone anaesthesia and increases the toxicity to strychnine shows that 48/80 has some action in common with tranquillizers and atropine-like substances, since these also prolong barbiturate sleep and increase strychnine toxicity (Sherman, 1956; Berger, Hendley and Lynes, 1956; Berger, 1957). This common mode of action might also explain why these substances aggravate the delirium ambulatorium and increase the mortality of intracerebral 48/80.

These facts suggest that 48/80, although it stimulates and produces delirium, can provide a useful tool in the study of the mechanism of action of tranquillizers. The potentiation of the effects of 48/80 by chlorpromazine, reserpine, and promethazine indicates that the tranquillizing activity is a compromise between two opposing activities, stimulation and sedation, upon different sites in the brain.

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