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ANTICONVULSANT COMPOUNDS AND 5-HYDROXY-TRYPTAMINE IN RAT BRAIN

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In rats, a series of anticonvulsant compounds have been shown to cause a significant elevation of brain 5-hydroxytryptamine (5-HT) levels in comparison with control values. This increase in 5-HT only occurred in brain tissue and was not observed in spleen, upper small intestine or blood. Elevation of brain levels of 5-HT by iproniazid (Marsilid) or 5-hydroxytryptophan failed to give protection against the convulsant or lethal action of lept zol (75 mg./kg.).

The effect of phenytoin (diphenylhydantoin, Dilantin) in preventing the response of the pituitary-adrenal system to various standard procedures, known to cause a discharge of the adrenocorticotrophic hormone (ACTH) and a resultant fall in adrenal ascorbic acid, has been studied both for a direct action of the drug upon the adrenal gland itself and a possible alteration in some central mechanism (Bonnycastle and Bradley, 1956, 1957). As it appeared fairly clear that, initially at least, the interference with the response is central in origin since the adrenal cortex remains normal in its responsiveness to exogenous trophic hormone, it was of interest to examine the levels of one or other of the suggested transmitting substances in the brain. In a preliminary report (Bonnycastle, Paasonen and Giarman, 1956) the 5-hydroxytryptamine (5-HT) content of the brains of rats treated with phenytoin was shown to be elevated approximately twofold over that of untreated control animals. The present paper deals with the brain levels of 5-HT in rats treated with other anti-epileptic drugs in use. Unlike phenytoin, none of these substances has any inhibitory effect upon the pituitary-adrenal system.

METHODS

Male rats of the Sprague-Dawley strain weighing 100 to 300 g. were used in these experiments. The compounds listed in Table I were examined. While the dosage varied considerably, an attempt was made to use doses which have been reported to be anticonvulsant in the rat against one or other convulsant procedure.

Since many of the substances used are insoluble in any suitable media, some problems of uniform administration arose. After some preliminary experiments, the most satisfactory method was to weigh out the individual doses and suspend them as microcrystalline preparations for intraperitoneal injection. No attempts were made to determine the minimal effective dose or the duration of effect. The original dosage regimen was based on the previous study (Bonnycastle and Bradley, 1956) and usually consisted of four doses given over two days, the animals being

TABLE I

EFFECT OF SOME ANTICONVULSANT COMPOUNDS UPON THE CONCENTRATION OF 5-HYDROXYTRYPTAMINE IN RAT BRAIN

The numerals in brackets in col. (3) indicate number of doses given. The difference between the values for the treated and control rats was in each instance, highly significant ($P<0.001$). The numerals in brackets in col. (6) give the standard error.

Drug		Individual Dose (mg./kg.)	Time after Last Dose (hr.)	No. of Rats	Conc. in Brain of 5-HT (ng./g.)
Chemical Name (1)	Name or Synonym (2)	(3)	(4)	(5)	(6)
Control				20	341 (±10·5)
5, 5 diphenyl hydantoin	Phenytoin Dilantin	100(16)	12	5	703 (±85)
5 ethyl, 3 methyl, 5 phenylhydantoin	Methion Mesantoin	200 (4)	12	3	607 (±46)
3, 5, 5 trimethyl-2, 4 oxazolidinedione	Troxidone Tridione	150 (4)	12	9	481 (±13.7)
3, 5 dimethyl 5 ethyloxazolidine, 2, 4, dione	Parametha-dione Paradione	200 (4)	12	3	501 (±10·0)
5, 5 diphenyloxazolidine, 2, 4 dione	Epidon	400 (4)	12	2	508 (±12·5)
N-methyl phenyl succimide	Phensux-amide Milontin	450 (4)	12	4	574 (±27)
Phenylacetyl urea	Phenace-mide Phenurone	500 (4)	12	6	561 (±20)
5 phenyl, 7 ethyl hexahydropyrimidine, 4, 6 dione	Primidone Mysoline	500 (4)	12	4	546 (±7·8)
5 ethyl-5 phenyl barbituric acid	Phenobarbi-tone	125 (1)	2·5	5	532 (±34·5)
5 phenyl-5-ethyl-3 methyl barbituric acid	Prominal Gemonil Mebaral	125 (1)	2·5	4	524 (±28·8)
Sodium bromide		1,000 (5)	4	4	531 (±38)

killed by decapitation 12 hr. after the last dose. In some instances, as noted in Table I and in the text, there has been a departure from this design.

The brains (without cerebella) were quickly removed and homogenized with an equal volume of isotonic saline in a Potter-Elvehjem type homogenizer. Extracts were then made of the total homogenates by the method of Amin, Crawford, and Gaddum (1954). When it was desired to determine the 5-HT content of tissues other than brain, these were first minced with scissors in saline, then homogenized and extracted in the same manner. The 5-HT content of the various extracts was determined by bioassay, using the heart of the mollusc *Venus mercenaria* suspended in sea-water in a 4 ml. over-flow type bath. The sea-water contained benzoquinonium (Mytolon) in a concentration of 6 mg./l., to prevent interference by choline esters in the extracts. This method of assay, introduced by Twarog and Page (1953) and used extensively by Gaddum and Paasonen (1955), has in our hands yielded reliable and reproducible estimates of 5-HT in biological tissues in the range of 1 to 10 ng.

The significance of the difference between levels found in the treated animals and the control figure is determined by Student's *t* test.

Results

In Table I it is seen that treatment of rats with various anticonvulsant drugs led to a significant increase in the brain levels of 5-HT. This increase was measured 12 hr. after the last dose, except in the case of sodium bromide and phenobarbitone and its methyl derivative. In view of the rather slow displacement of chloride by bromide a single daily dose was given for five days and the animals examined 4 hr. after the last dose. No change in brain 5-HT was observed 12 hr. after a single dose of phenobarbitone or the methyl derivative (325 to 380 ng./g. and 330 to 350 ng./g. respectively). When the 5-HT content was determined in rat brains 2.5 hr. after administration of a single dose of either of these barbiturates, it was found to be increased. These results suggested the possibility that only one dose was necessary with other drugs. It was found that methion (Mesantoin), paramethadione (Paradione), phensuxamide (Milontin), phenacemide and primidone all produced elevated values of brain 5-HT 12 hr. after a single dose of the size indicated in Table I.

The question was raised whether this action of the anticonvulsant drugs was limited solely to brain tissue, or whether their administration would also affect the 5-HT levels of other tissues. Table II presents the results of single experiments in which estimates were made of the effect of five of these compounds on venous blood (vena cava), small intestine and spleen. The results clearly indicate that no alteration occurred in the 5-HT content of tissues other than brain.

Table II

5-HYDROXYTRYPTAMINE CONTENT OF SOME BODY TISSUES OF RATS TREATED WITH ANTICONVULSANT AGENTS COMPARED WITH CONTROLS

Each set of results is derived from a single rat. The numerals in brackets indicate number of doses.

Treatment	Dose (mg./kg.)	Time after Last Dose (hr.)	Brain (ng./kg.)	Spleen (ng./kg.)	Small Intestine (ng./kg.)	Venal Cava Blood (ng./kg.)
None ..			340	8,100	4,800	780
,, ..			360	4,600	6,600	400
,, ..			450	3,700	3,800	450
,, ..			420	3,700	3,100	430
Phenytoin ..	100 (7)	12	580	3,200	2,900	400
Phenacamide	450 (1)	12	710	4,600	2,400	420
Phensuxamide	400 (1)	12	740	6,400	2,700	430
Parametha-dione	400 (1)	2·5	520	4,900	2,900	400
Phenobarbi-tone	125 (1)	2·5	710	5,500	4,900	520

In attempting to explain the elevation of levels of brain 5-HT by the anticonvulsant drugs, it seemed of some interest to determine whether the action might be due to inhibition of monoamine oxidase. With rat liver homogenates and tyramine as a substrate, it was not possible to demonstrate manometrically any such inhibition by phenytoin. Similarly phenytoin in amounts of 0.1 to 0.2 mg./ml. failed to influence the destruction by rat liver homogenates of added 5-HT. In addition, preliminary experiments by differential centrifuging have indicated no striking alteration of the normal pattern of distribution of 5-HT within rat brain cells after treatment with phenytoin or phenacemide.

The finding that anticonvulsant compounds led to an increase in brain 5-HT made it of interest to measure what effect, if any, this elevation would have upon the convulsant activity of leptazol (75 mg./kg.). Some rats were treated with iproniazid (100 mg./kg. of base) 11 hr., and others with 5-hydroxytryptophan (100 mg./kg.) 30 min. before administration of the above dose of leptazol. The results are given in Table III, from which it is clear that the elevated brain levels of 5-HT (which

Table III

CONVULSANT ACTION OF LEPTAZOL IN RATS PREVIOUSLY TREATED WITH IPRONIAZID (MARSILID) OR WITH 5-HYDROXYTRYPTOPHAN

	No. Showing Convulsions	Mortality	Survival Time (Min.)	
			Mean	Range
Control group ..	7/10	6/10	16	(5 to 30)
Iproniazid-treated group	7/10	5/10	56	(10 to 120)
5-hydroxytryptophan-treated group ..	6/10	6/10	16	(5 to 45)

is known from previous work to be about 800 ng./g. in the case of iproniazid, and 925 ng./g. in the case of 5-hydroxytryptophan) afforded little protection against the convulsant action of leptazol. The survival times of the animals that died in the treated groups showed some differences from the control group. Two of the deaths in the iproniazid group occurred 2 hr. after the administration of leptazol, while all those in the 5-hydroxytryptophan group occurred during the first 45 min.

Studies with meprobamate (Miltown), which has been said to exert a beneficial effect in some forms of epilepsy, such as petit mal (Perlstein, 1956), gave no indication of an alteration in the brain levels of 5-HT in the rat when examined about 2 hr. after administration. The brain 5-HT concentrations in treated rats were 322, 435, 334, and 329 ng./g. compared with a control of 341 ng./g. On the other hand, pentobarbitone in anaesthetic doses of 50 mg./kg. resulted in an elevation of brain levels of 5-HT after 45 min. to 524, 592, 565, and 540 ng./g. compared with a control value of 341 ng./g. Treatment with hexobarbitone (Evipan) in an initial anaesthetic dose of 100 mg./kg. with two supplements resulted in levels of 518 and 485 ng./kg. in the two cases which were examined after 45 min. These findings might have been expected in view of the results obtained with phenobarbitone and its methyl derivative, but nevertheless they raised the question of a possible association between the elevation of brain 5-HT and the action of the central depressant drugs in general. It was clear from the experiments carried out so far that the time at which these effects were seen differed with different drugs, and some of the apparent negative findings which have been observed may well be due to the fact that the examination was made at the wrong time.

Discussion

Very few compounds have been discovered which alter the brain levels of 5-HT. It is known that reserpine and the related substances rescinnamine and desoxyreserpine cause all tissue stores of 5-HT to be depleted. Only two types of substance have been reported to cause an elevation of tissue levels of 5-HT. Inhibition of monoamine-oxidase apparently permits some accumulation of 5-HT to take place, and the inhibitor most commonly used for this purpose is iproniazid (Marsilid). The other compound which has been shown to raise the tissue levels of 5-HT is its immediate precursor, 5-hydroxytryptophan The findings obtained in the present study are of some interest, for here there are a number of pharmacologically related compounds, all of which lead to an increase in the brain level of 5-HT in the rat. From the results obtained it appears probable that this increase is not merely the result of repeated dosage of the drugs, but can occur following a single dose of any one of the drugs, provided that it is sought at the appropriate time. No attempt was made to ascertain the minimal effective dose in any case, but it is very probable that they are lower than the figures reported in Table I. In only one instance was any examination made of the time course of the effect, and this was with phenobarbitone with which no appreciable alteration from control levels was detectable at 1.5 to 2 hr. (390 to 400 ng./g.) and 12 hr. (325 to 380 ng./g.) after treatment, but values as high as 600 ng./g. were found 2.5 hr. after treatment.

The existence of a relationship between an increase in brain 5-HT and the anticonvulsant action of these drugs is certainly open to question. It has been observed in rats that such convulsing procedures as electro-shock, or the administration of leptazol, picrotoxin and CO_2, do not change the brain levels of 5-HT at least under the conditions of the experiments (Paasonen and Giarman, unpublished observations; Bonnycastle, unpublished observations; Giarman and Golden, unpublished observations). The results in Table III make it clear that elevation of brain 5-HT obtained either by monoamine-oxidase inhibition or by supplying the precursor afforded no protection against the convulsant action of leptazol (75 mg./kg.). Although there was a somewhat longer survival time in the iproniazid-treated group, the ultimate mortality rate was similar in the three groups of animals. It will be of interest to determine the effect of some of these anticonvulsant compounds upon the brain levels of 5-HT in other species, particularly those in which it is possible to dissect discrete neural structures. It will also be of interest to determine the time relationships of the changes in brain levels of 5-HT and the anticonvulsant activity of some of these drugs.

The results given in Table II serve a dual purpose. They show that the effects produced by the anticonvulsant drugs are restricted to the cerebral tissues, but also they serve as internal standards for the experiments. It is to be expected that some of the drug or drug metabolites would be distributed in tissues other than brain, and the fact that the values for 5-HT in the other tissues examined were within the limits of the control figures answers, in part at least, the question as

to what effect these substances might have upon the system used for assay. The control levels for spleen and upper small intestine reported here are higher than available figures obtained by others (Erspamer, 1954; Paasonen and Giarman, unpublished observations), and this is probably due to the method of preparing the extracts. In one of the studies (Paasonen and Giarman), the tissues were only minced with scissors before extraction with acetone, while in the present study tissues were first minced with scissors and then homogenized before extraction. It is probable that this procedure would allow a more complete extraction, and thus account for the higher values reported here for these tissues.

The mechanism by which these anticonvulsant compounds bring about the observed increase in brain 5-HT is not known, but preliminary studies suggest that it is not the result of monoamine-oxidase inhibition.

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