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A METHOD FOR ASSESSING THE EFFECTS OF DRUGS ON THE CENTRAL ACTIONS OF 5-HYDROXYTRYPTAMINE

BY

S. J. CORNE, R. W. PICKERING AND B. T. WARNER*

From the Department of Pharmacological Research, Parke, Davis & Co., Hounslow, Middlesex

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Injection of 5-hydroxytryptophan into mice produces a characteristic head-twitch. For a given period of observation this response may be assessed quantitatively either by observing the proportion of mice showing at least one head-twitch (a quantal response) or by counting the number of head-twitches for each mouse (a graded response). A method, based on the quantal response, of investigating the effect of centrally acting compounds on the head-twitch response is described. Evidence is presented that the head-twitches are due to a central action of 5-hydroxytryptamine formed by decarboxylation of 5-hydroxytryptophan. Head-twitches are potentiated by monoamine oxidase inhibitors and by phenytoin. Antagonists tested include an inhibitor of decarboxylase, antagonists of 5-hydroxytryptamine, some antihistamines, major tranquillizers and analgesic and sympathomimetic drugs. Drugs which neither potentiate nor inhibit the response include barbiturates and minor tranquillizers. The method may be valuable in the preliminary examination of compounds likely to have a central action.

We have observed that the administration of sufficiently large doses of 5-hydroxytryptophan to mice produces spontaneous irregularly occurring head-twitches, which effect does not appear to have been described before. This head-twitch resembles a strong pinna reflex involving the whole head of the animal but, unlike the pinna reflex, occurs without tactile stimulation. Experiments described in this paper show that this effect is probably due to a central action of 5-hydroxytryptamine, that it can be assessed quantitatively and that it can be used to study potentiators and antagonists of 5-hydroxytryptamine *in vivo*.

METHODS

Albino mice of either sex and weighing between 16 and 25 g were used and were usually kept in groups of ten during the experimental periods.

Pinna reflexes in mice were tested by stimulation of the external auditory meatus with a fine hair. A response was considered positive when a head-shake was elicited from either ear and negative when no head-shakes could be elicited from stimulation of both ears. Drugs were injected subcutaneously and the mice were tested at various times after injection until peak inhibition was obtained.

Extraction and estimation of brain 5-hydroxytryptamine was by the method of Bogdanski, Pletscher, Brodie & Udenfriend (1956). Pooled brains from three mice were used for extraction

* Present address: Science in General Management Ltd., Grosvenor House, High Street, Croydon, Surrey.

of whole brains and from six mice for brain-stems. The final solutions were activated at 290 mμ in an Aminco-Bowman spectrophotofluorometer and the fluorescence was measured at 540 mμ (wavelengths uncorrected for instrumental error).

Doses of drugs are in terms of active acid or base and the following were used: harmine, pheniprazine hydrochloride, tranylcypromine sulphate, iproniazid phosphate, phenytoin sodium, α-methyltryptamine hydrochloride, etryptamine (α-ethyltryptamine) hydrochloride, methyldopa (α-methyldopa), cyproheptadine, (+)-2-bromolysergic acid diethylamide (BOL 148), methysergide maleate (1-methyllysergic acid butanolamide bimaleinate; UML 491), diphenhydramine hydrochloride, promethazine hydrochloride, lysergic acid diethylamide (LSD 25), 1-benzyl-5-methoxy-2,*NN*-trimethyltryptamine (BAB), chlorpheniramine maleate, chlorprothixene, chlorpromazine hydrochloride, reserpine, amitriptyline hydrochloride, trifluoperazine dihydrochloride, perphenazine hydrochloride, thioridazine hydrochloride, desipramine (desmethylimipramine) hydrobromide, chlordiazepoxide (methaminodiazepoxide) hydrochloride, imipramine hydrochloride, etonitazene [1-(β-diethylaminoethyl)-2-(*p*-ethoxybenzyl)-5-nitrobenzimidazole hydrochloride; 20684-Ba], methadone hydrochloride, morphine sulphate, phencyclidine, codeine phosphate, pethidine hydrochloride, amphetamine sulphate, methylamphetamine hydrochloride, methyl phenidate hydrochloride, dopa (β-3,4-dihydroxyphenylalanine), noradrenaline bitartrate, isoprenaline hydrochloride, amylobarbitone sodium, hexobarbitone sodium, pentobarbitone sodium, phenobarbitone sodium, meprobamate, mebutamate (2,2-dicarbamoyloxymethyl-3-methylpentane), mephenesin, azacyclonol hydrochloride, ethosuximide (ethylmethylsuccinimide), phensuximide, mepyramine maleate, 1-benzyl-5-methoxy-2-methyltryptamine (BAS; benanserin), 2,2-diethylpropane-1,3-diol (Prenderol) and 5-hydroxytryptophan.

Statistical methods. The analysis of quantal responses was based on the standard methods described by Finney (1952), using log dose as the dose metameter and logit transformation for the proportions. Where possible, the nomograms and tables of Berkson (1960) were used to estimate the ED50 value, its approximate 95% confidence limits and the slope of the fitted line. In the analysis of responses based on counts of head-twitches, standard linear regression methods for graded responses were used.

RESULTS

Head-twitch experiments

When administered to mice, 5-hydroxytryptamine in doses up to 200 mg/kg, L-tryptophan up to 300 mg/kg, dopa up to 600 mg/kg, dopamine up to 800 mg/kg and noradrenaline up to 5 mg/kg failed to produce head-twitches. Head-twitches similar to those produced by 5-hydroxytryptophan were occasionally observed in a small proportion of mice treated with saline, particularly during a short period immediately after placing the mice into a fresh box. However, at the time of maximum effect of 5-hydroxytryptophan, less than 1% of mice treated with saline exhibited head-twitches.

Head-twitches were produced by the administration of 5-hydroxytryptophan by the intraperitoneal, intravenous or subcutaneous routes, but in all experiments described here this compound was given intraperitoneally.

Fig. 1 shows the results of an experiment in which various doses of 5-hydroxytryptophan were administered to six groups of ten mice. The numbers of mice exhibiting at least one head-twitch during an observation period of 2 min were recorded at various times after the drug was administered. The peak activity occurred between 15 and 25 min, and this was confirmed by the ED50 values, which were 195 mg/kg at 5 min, 102 mg/kg at 15 min, 100 mg/kg at 25 min and 147 mg/kg at 35 min after administration of the drug.

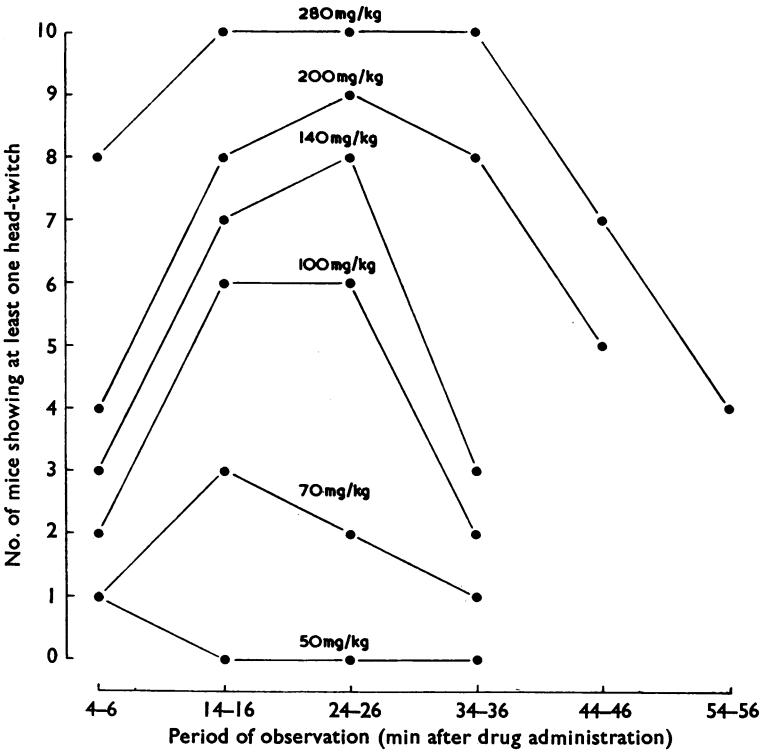

Fig. 1. Time-course of head-twitch response to 5-hydroxytryptophan (doses above lines) given intraperitoneally to mice in groups of ten.

Statistical analysis. For further work with the head-twitch, it was necessary to choose the length of the observation period and the response metameter. Some of the possibilities are illustrated by an experiment in which the number of head-twitches was counted for each mouse separately for each minute from 20 to 25 min after administration of 5-hydroxytryptophan.

TABLE 1

EFFECT OF THE LENGTH OF OBSERVATION PERIOD ON THE INCIDENCE OF HEAD-TWITCHES AFTER 5-HYDROXYTRYPTOPHAN

Observation periods were centred on 22 or 22·5 min. after intraperitoneal injection of 5-hydroxytryptophan

Dose of 5-hydroxytryptophan (mg/kg)	Number of mice (out of 10) showing at least one head-twitch in an observation period of				
	1 min	2 min	3 min	4 min	5 min
50	0	0	1	2	2
70	1	3	4	5	6
100	2	4	4	6	7
140	6	8	9	10	10
200	8	8	9	9	9
280	10	10	10	10	10
ED50 (mg/kg)	132	106	92	75	69
Approximate 95% limits of ED50 (mg/kg)	111–158	87–130	75–113	59–95	55–88
Slope (logits/$\log_{10}$ dose)	10·2	8·0	7·8	7·5	7·6

The proportion of mice showing one or more head-twitches during the chosen observation period is a possible criterion of response. This is a quantal response, and the ED50 values for different periods of observation are given in Table 1. For periods of 2 min or more the slopes of the log dose/response lines were similar, and the higher slope for the period of 1 min appears to be an artefact as the slopes for other 1 min periods during the experiment were lower and similar to those for the longer periods of observation. As the duration of observation increased, the ED50 value decreased. Statistical analysis showed that none of the log dose/response curves deviated significantly from linearity ($P>0.25$).

An alternative criterion of response is the number of head-twitches exhibited by each mouse. This is a graded response and is illustrated for a period of 2 min in Fig. 2 (similar results were obtained for other periods of observation from 1 to 5 min). In the left-hand diagram the log dose/response curve is very sigmoid and the standard error tends to increase with the mean count. With such non-linearity and heterogeneity of variation, standard linear regression methods may be misleading. The defects almost disappear if we analyse a head-twitch score (z) which is defined in terms of the count (y) by the formula

$$z=\log (1+10y).$$

It will be seen that the log dose/response curve based on the score is effectively linear and that, apart from the lowest dose where no head-twitches were observed, the standard errors are nearly constant.

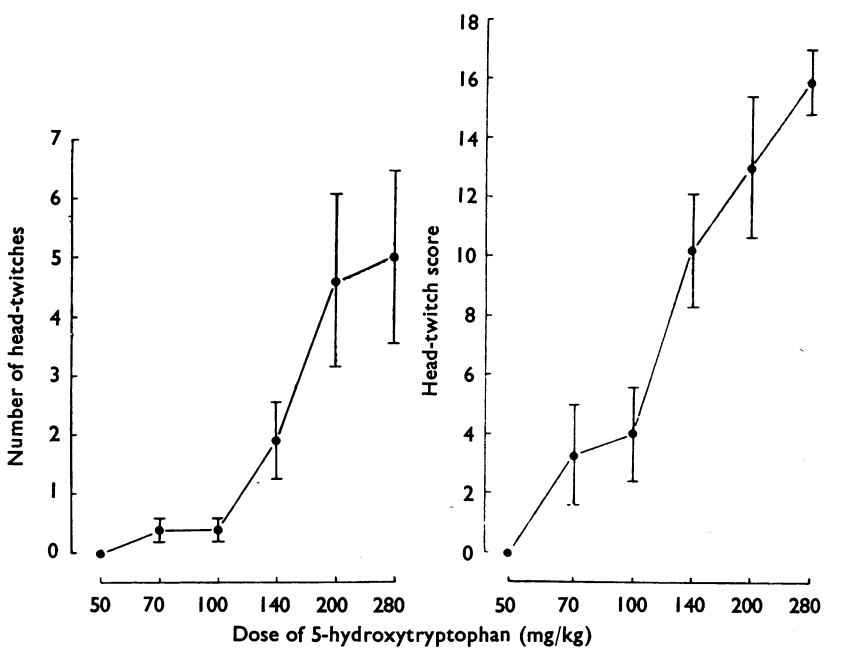

Fig. 2. Results with the use of number of head-twitches and head-twitch score as response metameters. Mice were observed for 2 min commencing 21 min after intraperitoneal injection of 5-hydroxytryptophan. Plotted points represent means for ten mice; vertical lines indicate standard errors of means. For a definition of head-twitch score, see text.

TABLE 2

EXPERIMENTS WITH 5-HYDROXYTRYPTOPHAN

Mice were observed for 2 min commencing 23 min or 24 min after intraperitoneal injection of 5-hydroxytryptophan. The experiments were carried out with different batches of mice on different days

Dose of 5-hydroxytryptophan (mg/kg)	Number of mice (out of 10) showing at least one head-twitch					
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	Expt. 6
50	1	—	—	—	0	1
70	—	2	1	0	2	4
100	4	2	3	2	6	3
140	—	6	4	4	8	8
200	8	8	7	9	9	8
280	—	—	—	—	10	9
ED50 (mg/kg)	117	129	150	141	100	105
Approximate 95% limits of ED50 (mg/kg)	83–165	100–168	112–204	121–168	83–119	81–136
Slope (logits/$\log_{10}$ dose)	6·0	7·0	6·2	12·7	10·0	5·6

Either the quantal or graded response could have been used for further work but, for reasons discussed later, it was decided to use the quantal response, with a period of observation of 2 min at about 25 min after giving 5-hydroxytryptophan. Table 2 gives the results of six experiments with 5-hydroxytryptophan. Statistical analysis shows that the log dose/response lines can be regarded as linear and parallel, and that the ED50 values are not significantly different at the 5% level. As a preliminary to further experiments, the probabilities of responses with 50 mg/kg and 300 mg/kg of 5-hydroxytryptophan were estimated. From the fitted log dose/response lines, the average percentages of mice responding to doses of 50 mg/kg and 300 mg/kg were 5.7% and 94.6% respectively.

Chance variation in the six experiments analysed and differences between ED50 values might lead to deviations from these estimated percentages. If it is assumed that the experiments of Table 2 are a random sample of all possible experiments, the upper 95% confidence limit of the percentage of mice responding to 50 mg/kg of 5-hydroxytryptophan in a further experiment is 16%, and the lower 95% confidence limit of the percentage of mice responding to 300 mg/kg of 5-hydroxytryptophan is 84%. These results justify the use of these doses to investigate potentiators and inhibitors of head-twitches induced by 5-hydroxytryptophan.

Effects of centrally acting compounds on head-twitches due to 5-hydroxytryptophan

Many compounds were examined in preliminary experiments to determine the times of peak effects on the head-twitch. In subsequent experiments the times at which the compounds were injected were arranged so that the observation period (25 min after 5-hydroxytryptophan) was at this time of peak effect.

Potentiating drugs. Table 3 shows the results of two experiments in which different doses of harmine were injected subcutaneously 15 min before 50 mg/kg of 5-hydroxytryptophan was injected intraperitoneally. In control groups of ten mice, no mouse showed head-twitches when 10 mg/kg of harmine was followed by saline, and when saline was followed by 50 mg/kg of 5-hydroxytryptophan.

TABLE 3

POTENTIATION OF HEAD-TWITCH RESPONSE TO 5-HYDROXYTRYPTOPHAN BY HARMINE

Harmine was injected subcutaneously 15 min before 50 mg/kg of 5-hydroxytryptophan intraperitoneally. Mice were observed for 2 min commencing 24 min after 5-hydroxytryptophan

Dose of harmine (mg/kg)	Number of mice (out of 10) showing at least one head-twitch	
	Expt. 1	Expt. 2
0·0	0	0
0·156	2	1
0·312	4	4
0·625	5	6
1·25	7	7
2·5	8	9
5	9	10
10	10	—
ED50 (mg/kg)	0·55	0·52
Approximate 95% limits of ED50 (mg/kg)	0·31–0·98	0·32–0·83

TABLE 4

COMPOUNDS POTENTIATING THE HEAD-TWITCH RESPONSE TO 5-HYDROXYTRYPTOPHAN

Compounds were injected subcutaneously at various times before 50 mg/kg of 5-hydroxytryptophan (5-HTP) intraperitoneally. ED50 values were determined from the numbers of mice showing at least one head-twitch during an observation period of 2 min commencing 24 min after 5-hydroxytryptophan

Compound	ED50 (95% limits) (mg/kg)	Time injected before 5-HTP
Harmine	0·55 (0·31–0·98)	15 min
	0·52 (0·32–0·83)	15 min
Pheniprazine	0·60 (0·39–0·92)	60 min
Tranylcypromine	1·0 (0·8–1·3)	60 min
α-Methyltryptamine	1·1 (0·8–1·6)	60 min
Etryptamine	1·5 (1·1–2·2)	60 min
Iproniazid	36·0 (18–71)	24 hr
Phenytoin	84·0 (69–102)	15 min

TABLE 5

INHIBITION OF HEAD-TWITCH RESPONSE TO 5-HYDROXYTRYPTOPHAN BY METHYLDOPA

Methyldopa was injected subcutaneously 60 min before 300 mg/kg of 5-hydroxytryptophan intraperitoneally. Mice were observed for 2 min commencing 24 min after 5-hydroxytryptophan

Dose of methyldopa (mg/kg)	Number of mice (out of 10) showing at least one head-twitch	
	Expt. 1	Expt. 2
0	9	
25	7	7
50	5	4
100	1	2
200	—	0
ED50 (mg/kg)	42	40
Approximate 95% limits of ED50 (mg/kg)	28–63	26–62

TABLE 6

COMPOUNDS INHIBITING THE HEAD-TWITCH RESPONSE TO 5-HYDROXYTRYPTOPHAN AND THEIR EFFECT ON THE PINNA REFLEX

In head-twitch experiments, compounds were injected subcutaneously at various times before 300 mg/kg of 5-hydroxytryptophan (5-HTP) intraperitoneally. ED50 values were determined from the numbers of mice showing at least one head-twitch during an observation period of 2 min commencing 24 min after 5-hydroxytryptophan. In pinna reflex experiments, compounds were injected subcutaneously and the pinna reflex tested at varying times after injection until the peak effect was obtained. ED50 values were determined, when possible, at this time. BOL 148 = (+)-2-bromolysergic acid diethylamide; LSD 25 = lysergic acid diethylamide; BAB = 1-benzyl-5-methoxy-2,*NN*-trimethyltryptamine

	Head-twitch		Pinna reflex		
Compound	ED50 (95% limits) (mg/kg)	Time injected before 5-HTP	ED50 (95% limits) (mg/kg)	Time of peak effect	ED50 Pinna reflex / ED50 Head-twitch (95% limits)
Decarboxylase inhibitor					
Methyldopa	42 (28–63)	60 min	>200		
	40 (26–62)	60 min			
Antagonists of 5-hydroxytryptamine and of histamine					
Cyproheptadine	0·30 (0·12–0·76)	30 min	>100		
BOL 148	3·8 (2·8–5·2)	30 min	>20		
Methysergide	4·1 (3·0–5·5)	30 min	>40		
Diphenhydramine	4·2 (1·5–11·5)	30 min	>100		
Promethazine	6·3 (4·3–9·2)	30 min	140 (100–190)	30 min	22 (14–36)
LSD 25	8·1 (5·5–11·7)	60 min	>20		
BAB	11 (9–14)	30 min			
Chlorpheniramine	60 (38–95)	15 min	>400		
Tranquillizers and related compounds					
Chlorprothixene	0·055 (0·030–0·101)	30 min	1·7 (1·0–2·7)	60 min	31 (14–68)
Chlorpromazine	0·86 (0·63–1·18)	30 min	2·3 (1·7–3·0)	60 min	2·7 (1·7–4·1)
Reserpine	1·3 (1·0–1·8)	24 hr	6·3 (3·3–12·0)	4 hr	4·8 (2·4–9·8)
Amitriptyline	1·4 (1·1–1·8)	30 min	120 (90–170)	30 min	86 (56–130)
Trifluoperazine	1·4 (1·0–2·0)	30 min	19 (11–31)	60 min	13 (7–25)
Perphenazine	5·0 (3·9–6·4)	30 min	57 (48–67)	120 min	11 (9–15)
Thioridazine	5·7 (4·5–7·3)	30 min	140 (70–280)	60 min	25 (12–51)
Desipramine	12 (5–29)	30 min	≏500	30 min	≏40
Chlordiazepoxide	36 (24–54)	30 min	>400		
Imipramine	59 (50–69)	30 min	130 (90–210)	30 min	2·3 (1·4–3·6)
Analgesics					
Etonitazene	0·011 (0·007–0·016)	30 min	0·022 (0·016–0·030)	30 min	2·0 (1·2–3·4)
Methadone	0·80 (0·54–1·17)	30 min	5·9 (4·2–8·3)	30 min	7·4 (4·4–12·3)
Morphine	1·6 (1·2–2·3)	30 min	16 (10–26)	30 min	10 (6–18)
Phencyclidine	2·8 (1·7–4·7)	30 min	7·1 (4·7–10·5)	30 min	2·5 (1·3–4·9)
Codeine	7·7 (5·1–11·6)	30 min	220 (130–370)	60 min	29 (15–55)
Pethidine	20 (15–28)	15 min	38 (33–43)	15 min	1·9 (1·3–2·6)
Sympathomimetic drugs and related compounds					
Amphetamine	5·6 (3·7–8·4)	30 min	>50		
Methylamphetamine	7·7 (5·8–10·5)	30 min	>40		
Methyl phenidate	14 (11–18)	30 min	>50		
Dopa	420 (320–560)	30 min			
Noradrenaline	3·2 (2·8–3·7)	19 min after 5-HTP	>5		
Isoprenaline	20 (10–38)	19 min after 5-HTP	>200		

Table 4 lists compounds that potentiated the head-twitch response. Apart from phenytoin, these compounds are known to be monoamine oxidase inhibitors, and the mice exhibited more general activity than after 5-hydroxytryptophan alone. With the larger doses of potentiator, tremors and generalized convulsions were often observed.

Inhibiting drugs. Table 5 shows the results of two experiments in which different doses of methyldopa were injected subcutaneously 60 min before 5-hydroxytryptophan was injected intraperitoneally. In control groups of ten mice, no mouse showed head-twitches when 100 mg/kg of methyldopa was followed by saline, and nine mice showed head-twitches when saline was followed by 300 mg/kg of 5-hydroxytryptophan. Table 6 lists compounds that inhibited the head-twitch response. They fall into five distinct classes. With the exception of diphenhydramine, chlorpheniramine, amphetamine and methylamphetamine, each of these antagonists produced either sedation or no obvious effect when 5-hydroxytryptophan was given. With chlorpheniramine and, to a lesser extent, with diphenhydramine, the mice became very active when 5-hydroxytryptophan was given. With amphetamine and with methylamphetamine, the activity normally produced by these compounds was not obviously affected after injection of 5-hydroxytryptophan. The compounds listed in Table 6 were also tested for their ability to inhibit the pinna reflex. The results are given in the same table.

Inactive compounds. Table 7 lists all the other compounds that we have tested and that neither potentiated nor inhibited the head-twitch response in the doses used.

TABLE 7

COMPOUNDS HAVING NO EFFECT ON THE HEAD-TWITCH RESPONSE TO 5-HYDROXYTRYPTOPHAN

Compounds were injected subcutaneously at various times before 50 mg/kg and 300 mg/kg of 5-hydroxytryptophan (5-HTP) intraperitoneally. BAS = 1-benzyl-5-methoxy-2-methyltryptamine

Compound	Maximum dose given (mg/kg)	Time injected before 5-HTP (min)
Amylobarbitone	100	30
Hexobarbitone	50	5
Pentobarbitone	20	30
Phenobarbitone	80	30
Meprobamate	1,000	15
Mebutamate	200	30
Mephenesin	200	15
Azacyclonol	200	30
Ethosuximide	100	30
Phensuximide	100	30
Mepyramine	40	30
BAS	56	30

Brain concentrations of 5-hydroxytryptamine after injection of 5-hydroxytryptophan

Whole brain and brain-stem concentrations of 5-hydroxytryptamine were determined at various times after intraperitoneal injection of 120 mg/kg of 5-hydroxytryptophan. For whole brains, the time courses of 5-hydroxytryptamine concentration and number of mice exhibiting head-twitches were not consistently

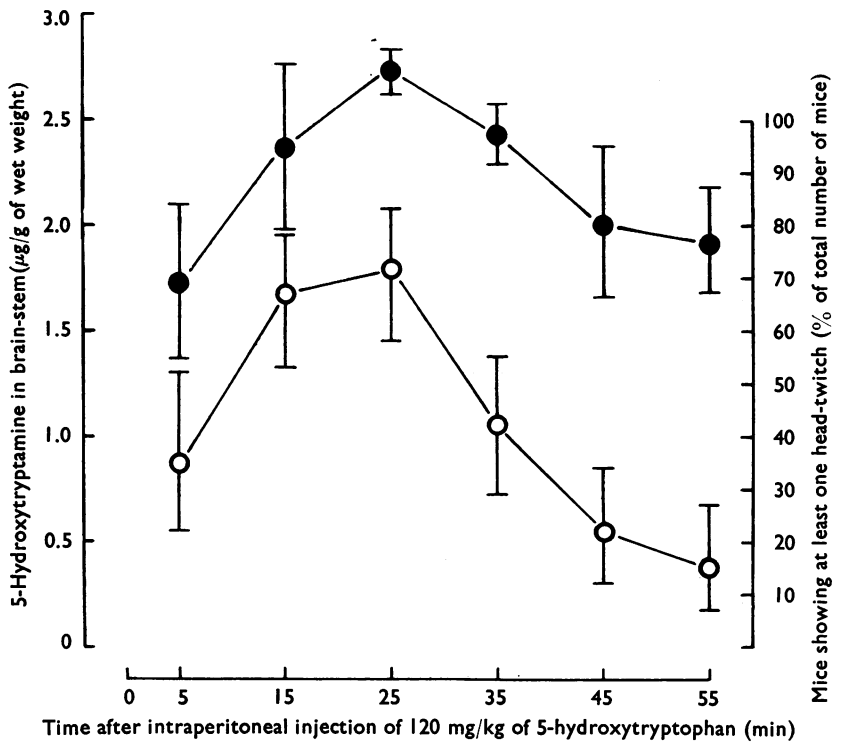

Fig. 3. Time-courses of brain-stem levels of 5-hydroxytryptamine (●—●) and of head-twitches (○—○) after intraperitoneal injection of 5-hydroxytryptophan (120 mg/kg). Concentrations of 5-hydroxytryptamine were obtained from pooled brain-stems of six mice from three experiments. The head-twitch percentages were obtained from six experiments each using a group of ten mice and observing them for 2 min periods at each time. The values given are the means with 95% fiducial limits.

parallel. Thus, although peak concentration and maximal head-twitch response were usually coincident 25 min after injection of 5-hydroxytryptophan, decrease in the number of the head-twitches was not necessarily followed by a fall in 5-hydroxytryptamine level. When the 5-hydroxytryptamine concentrations in the brain-stems were determined, however, the time courses of the two events were consistently parallel (Fig. 3).

DISCUSSION

As has been shown, either a quantal or a graded response can be used to assess quantitatively the head-twitch. A disadvantage of a quantal response is that it is generally less informative in a statistical sense than the underlying graded response, so that more mice per dose-group are needed ; this increases the expenditure on animals and on 5-hydroxytryptophan, an expensive compound. In work with the head-twitch, the quantal response has the advantage that ten mice may be observed simultaneously ; for the graded response, the head-twitches are counted and single mice must be observed. We chose to use the quantal response, but other workers may prefer the graded response.

The mice were observed 25 min after administration of 5-hydroxytryptophan because this was near the time of peak effect. The observation period of 2 min was chosen as it was convenient; a longer period may lead to observer fatigue, and too short a period gives a high ED50 so that higher doses of 5-hydroxytryptophan are necessary.

At an early stage in our work, two methods for investigating antagonists and potentiators of the head-twitch were considered. One was to determine the dose-ratio from the dose/response curve of 5-hydroxytryptophan in mice previously treated with a constant dose of antagonist or potentiator; this method was discarded because it did not facilitate direct comparisons of the potencies of antagonists or potentiators. The method adopted and described in this paper has the advantage that equi-active doses of compounds are directly determined, and hence relative potencies may be deduced.

The production of abnormal head-movements in animals has been described by a number of workers and the literature has been reviewed by Zettler, Müller & Warm (1959). In only one instance (Keller & Umbreit, 1956) was a head movement described similar to that obtained after injection of 5-hydroxytryptophan; the effect was a rapid head-shaking or twitch in response to a light touch at the back of the head of mice treated with lysergic acid diethylamide and of some mice that had been kept in solitary confinement for 3 weeks. We have been able to confirm only the results with isolated animals (Corne & Pickering, unpublished). Recently, Hess & Doepfner (1961) reported head-shaking after injection of tryptophan or 5-hydroxytryptophan into rats previously treated with a monoamine oxidase inhibitor, and Blum (1962) reported head-shaking in rats after 2,2-diethylpropane-1,3-diol. We have confirmed both these findings with rats (Corne & Pickering, unpublished). The head-shaking response after tryptophan or 5-hydroxytryptophan plus a monoamine oxidase inhibitor is similar to that in mice after 5-hydroxytryptophan alone, but the response after 2,2-diethylpropane-1,3-diol is unlike that after 5-hydroxytryptophan in rats and it cannot be elicited by this compound in mice.

It seems reasonable to infer that the head-twitch induced by 5-hydroxytryptophan in mice is due to some central action of 5-hydroxytryptamine because:

(*a*) It is produced after injection of 5-hydroxytryptophan and not after injection of 5-hydroxytryptamine. It has been shown that 5-hydroxytryptophan passes the blood-brain barrier but that 5-hydroxytryptamine does not (Udenfriend, Weissbach & Bogdanski, 1957);

(*b*) it is potentiated by monoamine oxidase inhibitors;

(*c*) it is antagonized after inhibition of decarboxylase and by specific antagonists of 5-hydroxytryptamine; and

(*d*) after injection of 5-hydroxytryptophan, the time-course of the head-twitch response corresponds with that of the brain-stem levels of 5-hydroxytryptamine (Fig. 3).

Presumably compounds which selectively modify the head-twitch response to 5-hydroxytryptophan do so by affecting the metabolism of 5-hydroxytryptophan

or 5-hydroxytryptamine or by altering or interfering with the central actions of 5-hydroxytryptamine.

With the exception of phenytoin, which will be discussed later, the compounds which potentiated the head-twitch are monoamine oxidase inhibitors. In the case of harmine, iproniazid, pheniprazine and tranylcypromine, their relative potencies agree well with those obtained by other methods (Ozaki, Weissbach, Ozaki, Witkop & Udenfriend, 1960; Maxwell, Gray & Taylor, 1961). On the other hand, α-methyltryptamine and etryptamine appear more potent than might be expected from their monoamine oxidase inhibitory activity (Greig, Walk & Gibbons, 1959); this may be due to their tryptamine- or 5-hydroxytryptamine-like activity (Vane, Collier, Corne, Marley & Bradley, 1961).

When dealing with antagonists of the head-twitch, it is useful to know whether the antagonist is generally depressing the central nervous system or is more specifically concerned with the metabolism or action of 5-hydroxytryptamine. To this end, we tested antagonists of the head-twitch response on the pinna reflex, which has a multi-neurone pathway (Sherrington, 1917). This reflex is useful in the study of drug action (Goodsell, Toman, Everett & Richards, 1954; Witkin, Spitaletta & Plummer, 1959). A compound which antagonizes the head-twitch in doses which have no effect on the pinna reflex probably has a selective effect on 5-hydroxytryptamine action or metabolism, whereas a compound which affects both the head-twitch and pinna reflex in similar doses probably has a non-selective effect and is more general in its depressant action.

Antagonists of the head-twitch have been divided into five broad classes according to their pharmacological actions. The only compound in the first class is methyldopa, which inhibits the decarboxylation of 5-hydroxytryptophan to 5-hydroxytryptamine. The total absence of effect on the pinna reflex in doses up to 200 mg/kg suggests that the action of methyldopa on the decarboxylase is probably the sole mechanism of its inhibition of the head-twitch.

Most of the compounds in the remaining four classes have been described as antagonists of 5-hydroxytryptamine (Gyermek, 1961), although it is unlikely that they all act by the same mechanism. In this respect, however, with the exception of promethazine, all the "specific" antagonists of 5-hydroxytryptamine and the antihistamines possessing some anti-5-hydroxytryptamine activity antagonize only the head-twitch and have no effect on the pinna reflex. These results agree with the generally accepted belief that these compounds antagonize 5-hydroxytryptamine at the receptor site. Promethazine, a phenothiazine derivative, does block the pinna reflex as well as the head-twitch and presumably possesses some of the properties of the third class of antagonists, the tranquillizers and related compounds.

The antagonism of 5-hydroxytryptamine by phenothiazines in general and chlorpromazine in particular is well documented (Gyermek, 1961). Gey & Pletscher (1961) have suggested that chlorpromazine and chlorprothixene (a thiaxanthene) act by decreasing the permeability of storage organelles for 5-hydroxytryptamine and noradrenaline, and Shore, Pletscher, Tomich, Carlsson, Kuntzman & Brodie (1957) found that reserpine impairs the ability of binding sites to take up 5-hydroxytrypt-

amine. Similarly, imipramine inhibits the uptake of 5-hydroxytryptamine by platelets *in vitro* and *in vivo* (Marshall, Stirling, Tait & Todrick, 1960 ; Stacey, 1961 ; Long & Lessin, 1962). Thus, an effect on the binding or an interference with the transport of 5-hydroxytryptamine, may account for the inhibition by drugs of this class, of the head-twitches induced by 5-hydroxytryptophan.

With the exception of chlorpromazine, reserpine and imipramine, compounds in this class inhibited the pinna reflex only in doses at least ten times greater than those required to inhibit the head-twitch. This implies that antagonism of the head-twitch response is produced mainly by an effect on 5-hydroxytryptamine action or metabolism or, as suggested above, on its uptake or binding rather than by the result of general nervous system depression. In fact, with the doses required to abolish the head-twitch, behavioural effects were slight whereas those doses required to affect the pinna reflex produced obvious neurological effects such as sedation. With chlorpromazine, reserpine and imipramine, however, effects on the head-twitch could not be dissociated from general central nervous system depression, although desipramine, which is believed to be the active metabolite of imipramine (Sulser, Watts & Brodie, 1962), does exhibit this differentiation to a marked degree. Doses up to 400 mg/kg of chlordiazepoxide had no effect on the pinna reflex, and in this respect the drug resembles the antagonists of 5-hydroxytryptamine and the antihistamines.

Medaković (1958) compared the peripheral antagonisms of 5-hydroxytryptamine by morphine-like analgesics and the order of potency (methadone>morphine> pethidine>codeine) parallels that in the pinna reflex experiments. However, in the head-twitch experiments, codeine is more active as an antagonist than pethidine. With the exception of codeine, the ratios between head-twitch and pinna reflex ED50 values are rather low, which suggests that the inhibitory action of the drugs on the head-twitch is closely related to their ability to inhibit spinal reflexes. Codeine, however, resembles more the tranquillizers and related compounds.

Although phencyclidine has no analgesic action in mice, it is included with the analgesic class of drugs in Table 6 because it has analgesic actions in man.

A possible functional role for the mutual antagonism of 5-hydroxytryptamine and sympathomimetic amines in the central nervous system has been proposed by Brodie, Spector & Shore (1959). Gaddum & Vogt (1956) have shown that amphetamine and methylamphetamine antagonize the central actions of 5-hydroxytryptamine when these compounds are injected into the cerebral ventricles of cats. It is, therefore, of interest that sympathomimetic amines antagonize the head-twitches produced by 5-hydroxytryptophan. Relatively large doses of noradrenaline and isoprenaline are required, probably because these compounds do not readily pass the blood-brain barrier. Dopa, which is included in this class of antagonist because it is a precursor of noradrenaline, may also act by competing with 5-hydroxytryptophan for the decarboxylase. These compounds have no effect on the pinna reflex.

The list of compounds having no effect on the head-twitch induced by 5-hydroxytryptophan shows that general central nervous system depression alone cannot account for the antagonism of the response. Bonnycastle, Paasonen & Giarman (1956) and Bonnycastle, Bonnycastle & Anderson (1962) have reported that several central

depressants, including barbiturates, morphine and phenytoin, raise the brain-levels of 5-hydroxytryptamine in rats. If these compounds have a similar effect on 5-hydroxytryptamine levels in mice, then there is a paradoxical situation that phenytoin is a potentiator and morphine an antagonist of the head-twitch response, whilst barbiturates neither potentiate nor antagonize it. We have shown, however, that the head-twitches are probably related to brain-stem levels of 5-hydroxytryptamine rather than whole brain levels, which Bonnycastle *et al.* (1956, 1962) measured. Further, Schanberg & Giarman (1962) have shown that behavioural changes in rats produced by various centrally acting drugs (reserpine, chlorpromazine, phenobarbitone, lysergic acid diethylamide, imipramine, iproniazid and pheniprazine) were correlated with changes between the relative proportions of free and bound 5-hydroxytryptamine rather than changes in absolute levels.

Table 7 shows that the minor tranquillizers, meprobamate and glutethimide, differ from the major ones in not antagonizing the head-twitch. 1-Benzyl-5-methoxy-2-methyltryptamine (Shaw & Woolley, 1956a), which has been reported to have a tranquillizing action in animals (Rudy, Costa, Rinaldi & Himwich, 1958), was inactive against the head-twitches, whereas the related compound, 1-benzyl-5-methoxy-2,*NN*-trimethyltryptamine (Shaw & Woolley, 1956b), was a moderately active antagonist (Table 6). It is noteworthy that of all the antihistamines examined only mepyramine failed to show any definite antagonism towards the head-twitch. This may indicate its high specificity.

Our results, with the exception of those pertaining to morphine and reserpine, are in close agreement with those of Tedeschi, Tedeschi & Fellows (1959, 1960, 1961), who used tryptamine convulsions in rats as an indication of central 5-hydroxytryptamine activity. These workers found that neither morphine nor reserpine antagonized tryptamine convulsions. There is good evidence to show that tryptamine and 5-hydroxytryptamine act on similar receptors in the central nervous system (Tedeschi *et al.*, 1959; Vane *et al.*, 1961) so these differences cannot as yet be accounted for.

The head-twitch response to 5-hydroxytryptophan in mice provides a simple method of determining the potencies of potentiators and antagonists of the central actions of 5-hydroxytryptamine. Any explanations why such a wide variety of pharmacological agents should be active in this test must be speculative at present. Nevertheless, we believe that this test is valuable in the preliminary examination of compounds likely to have a central action.

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REFERENCES

BERKSON, J. (1960). Nomograms for fitting the logistic function by maximum likelihood. *Biometrika*, **47**, 121–141.

BLUM, B. (1962). Head-shaking and nystagmus produced by 2,2-diethyl-1,3-propanediol (Prenderol) in the rat. *Arch. int. Pharmacodyn.*, **137**, 128–136.

BOGDANSKI, D. F., PLETSCHER, A., BRODIE, B. B. & UDENFRIEND, S. (1956). Identification and assay of serotonin in brain. *J. Pharmacol. exp. Ther.*, **117**, 82–88.

BONNYCASTLE, D. D., BONNYCASTLE, M. F. & ANDERSON, E. G. (1962). The effect of a number of central depressant drugs upon brain 5-hydroxytryptamine levels in the rat. *J. Pharmacol. exp. Ther.*, **135**, 17–20.

BONNYCASTLE, D. D., PAASONEN, M. H. & GIARMAN, N. J. (1956). Diphenylydantoin and brain levels of 5-hydroxytryptamine. *Nature (Lond.)*, **178**, 990–991.

BRODIE, B. B., SPECTOR, S. & SHORE, P. A. (1959). Interaction of drugs with norepinephrine in the brain. *Pharmacol. Rev.*, **11**, 548–564.

FINNEY, D. J. (1952). *Statistical Method in Biological Assay*, pp. 437–490. London: Griffin.

GADDUM, J. H. & VOGT, M. (1956). Some central actions of 5-hydroxytryptamine and various antagonists. *Brit. J. Pharmacol.*, **11**, 175–179.

GEY, K. F. & PLETSCHER, A. (1961). Influence of chlorpromazine and chlorprothixene on the cerebral metabolism of 5-hydroxytryptamine, norepinephrine and dopamine. *J. Pharmacol. exp. Ther.*, **133**, 18–24.

GOODSELL, J. S., TOMAN, J. E. P., EVERETT, G. M. & RICHARDS, R. K. (1954). A search for more effective muscle relaxants among the glycerol ethers and dioxolanes. *J. Pharmacol. exp. Ther.*, **110**, 251–259.

GREIG, M. E., WALK, R. A. & GIBBONS, A. J. (1959). The effect of three tryptamine derivatives on serotonin metabolism *in vitro* and *in vivo*. *J. Pharmacol. exp. Ther.*, **127**, 110–115.

GYERMEK, L. (1961). 5-Hydroxytryptamine antagonists. *Pharmacol. Rev.*, **13**, 399–439.

HESS, S. M. & DOEPFNER, W. (1961). Behavioural effects and brain amine content in rats. *Arch. int. Pharmacodyn.*, **135**, 89–99.

KELLER, D. L. & UMBREIT, W. W. (1956). Permanent alteration of behaviour in mice by chemical and physiological means. *Science*, **124**, 723–724.

LONG, R. F. & LESSIN, A. W. (1962). Inhibition of 5-hydroxytryptamine uptake by platelets *in vitro* and *in vivo*. *Biochem. J.*, **82**, 4–5*P*.

MARSHALL, E. F., STIRLING, G. S., TAIT, A. C. & TODRICK, A. (1960). The effect of iproniazid and imipramine on the blood platelet 5-hydroxytryptamine level in man. *Brit. J. Pharmacol.*, **15**, 35–41.

MAXWELL, D. R., GRAY, W. R. & TAYLOR, E. M. (1961). Relative activity of some inhibitors of monoamine oxidase in potentiating the action of tryptamine *in vitro* and *in vivo*. *Brit. J. Pharmacol.*, **17**, 310–320.

MEDAKOVIĆ, M. (1958). Comparisons of antagonistic potencies of morphine-like analgesics towards 5-hydroxytryptamine on the guinea-pig ileum. *Arch. int. Pharmacodyn.*, **114**, 201–209.

OZAKI, M., WEISSBACH, H., OZAKI, A., WITKOP, B. & UDENFRIEND, S. (1960). Monoamine oxidase inhibitors and procedures for their evaluation *in vivo* and *in vitro*. *J. med. pharm. Chem.*, **2**, 591–607.

RUDY, L., COSTA, E., RINALDI, F. & HIMWICH, H. E. (1958). Clinical evaluation of BAS (benzyl analogue of serotonin), a tranquillizing drug. *J. nerv. ment. Dis.*, **126**, 284–293.

SCHANBERG, S. M. & GIARMAN, W. J. (1962). Drug-induced alterations in the sub-cellular distribution of 5-hydroxytryptamine in rat's brain. *Biochem. Pharmacol.*, **11**, 187–194.

SHAW, E. N. & WOOLLEY, D. W. (1956a). Methylserotonins as potent antimetabolites of serotonin active both *in vitro* and *in vivo*. *J. Pharmacol. exp. Ther.*, **116**, 164–176.

SHAW, E. N. & WOOLLEY, D. W. (1956b). Benzyldimethylbufotenin, a powerful antimetabolite of serotonin. *Proc. Soc. exp. Biol. (N.Y.)*, **93**, 217–220.

SHERRINGTON, C. S. (1917). Reflexes elicitable in the cat from pinna vibrissae and jaws. *J. Physiol. (Lond.)*, **51**, 404–431.

SHORE, P. A., PLETSCHER, A., TOMICH, E. G., CARLSSON, A., KUNTZMAN, R. & BRODIE, B. B. (1957). Role of brain serotonin in reserpine action. *Ann. N.Y. Acad. Sci.*, **66**, 609–615.

STACEY, R. S. (1961). Uptake of 5-hydroxytryptamine by platelets. *Brit. J. Pharmacol.*, **16**, 284–295.

SULSER, F., WATTS, J. & BRODIE, B. B. (1962). On the mechanism of antidepressant action of imipramine-like drugs. *Ann. N.Y. Acad. Sci.*, **96**, 279–286.

TEDESCHI, D. H., TEDESCHI, R. E. & FELLOWS, E. J. (1959). The effects of tryptamine on the central nervous system, including a pharmacological procedure for the evaluation of iproniazid-like drugs. *J. Pharmacol. exp. Ther.*, **126**, 223–232.

TEDESCHI, D. H., TEDESCHI, R. E. & FELLOWS, E. J. (1960). *In vivo* monoamine oxidase measured by potentiation of tryptamine convulsions in rats. *Proc. Soc. exp. Biol. (N.Y.)*, **103**, 680–682.

TEDESCHI, D. H., TEDESCHI, R. E. & FELLOWS, E. J. (1961). Central serotonin antagonist activity of a number of phenothiazines. *Arch. int. Pharmacodyn.*, **132**, 172–179.

UDENFRIEND, S., WEISSBACH, H. & BOGDANSKI, D. F. (1957). Increase in tissue serotonin following administration of its precursor 5-hydroxytryptophan. *J. biol. Chem.*, **224**, 803–810.

VANE, J. R., COLLIER, H. O. J., CORNE, S. J., MARLEY, E. & BRADLEY, P. B. (1961). Tryptamine receptors in the central nervous system. *Nature (Lond.)*, **191**, 1068–1069.

WITKIN, L. B., SPITALETTA, P. A. & PLUMMER, A. J. (1959). Effects of some central depressants on two simple reflexes in the mouse. *J. Pharmacol. exp. Ther.*, **126**, 330–333.

ZETTLER, G., MÜLLER, W. & WARM, I. (1959). Jactatio Capitis eine pharmakologisch anagelöste motorische Stereotypie der Maus. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **237**, 247–263.