Neuropharmacology Vol. 22, No. 8, pp. 993–1000, 1983
Printed in Great Britain.

0028-3908/83 $03.00 + 0.00

THE HEAD-TWITCH RESPONSE TO INTRAPERITONEAL INJECTION OF 5-HYDROXYTRYPTOPHAN IN THE RAT: ANTAGONIST EFFECTS OF PURPORTED 5-HYDROXYTRYPTAMINE ANTAGONISTS AND OF PIRENPERONE, AN LSD ANTAGONIST

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(*Accepted* 28 *January* 1983)

Summary—The putative 5-hydroxytryptamine (5-HT) antagonists 2-bromo-LSD, cinanserin, cyproheptadine, pizotifen, methysergide, metitepine, mianserin and metergoline were found to reduce the frequency of the head-twitch response induced by intraperitoneal injections of 320 mg/kg of 5-hydroxytryptophan (5-HTP) in the rat. The antagonist dose–effect curve of these agents was biphasic. It consisted of an initial, steep, phase and a subsequent, shallower, phase. Analysis of the data by means of quantitative and quantal methods yielded different rank orders of potency of antagonist drugs. Only pirenperone, a drug identified earlier as a pure antagonist, produced a simple, monophasic dose–effect curve in antagonizing the effects of 5-HTP. The antagonist effects of pirenperone, and the first phase of the curve of the putative 5-HT antagonists, may reflect antagonist activity at 5-HT_2 receptors. The data are consistent with earlier behavioural evidence that the putative 5-HT antagonists act complexly as mixed agonist–antagonists; only pirenperone exerted behavioural effects that suggest it to be a pure antagonist.

Key words: head-twitch response, 5-hydroxytryptophan, 5-hydroxytryptamine, rat behaviour, pirenperone, agonist/antagonist.

Recent data (Colpaert, Niemegeers and Janssen, 1982) indicate that several drugs which are known as 5-hydroxytryptamine (5-HT) receptor blockers may act complexly as partial and mixed agonists/antagonists in a behavioural assay. In rats trained to discriminate intraperitoneal injections of 0.16 mg/kg of *d*-lysergic acid diethylamide (LSD) from saline injections, these drugs exerted both partial LSD-antagonist and partial LSD-like agonist effects. The dose–response curve of these agents in antagonizing the effects of LSD typically consisted of an initial, steep, rise and a subsequent, shallow, approach to a ceiling level of effect. This biphasic shape of the antagonist dose–response curve was interpreted to reflect the paradoxical agonist effects that these drugs produced at larger doses. The complex effects of putative 5-HT antagonists revealed by the LSD discrimination assay (Colpaert *et al.*, 1982), are not apparent, however, from earlier analyses of the behavioural actions of these agents (e.g. Green and Grahame-Smith, 1976; Jacobs, 1976; Matthews and Smith, 1980; Sloviter, Drust and Connor, 1978). These considerations have led to the effects of purported 5-HT receptor blocking agents being examined in some detail in a behavioural assay which is thought to involve activation of 5-HT receptors in brain.

Corne, Pickering and Warner (1963) reported that intraperitoneal injections of 5-hydroxytryptophan (5-HTP) produce a head-twitch response in mice. 5-Hydroxytryptophan is the immediate precursor of 5-HT. The response can similarly be produced by intracerebral administration of 5-HT itself (Suchowsky, Pegrassi, Moretti and Bonsignori, 1969) and considerable evidence has been accumulated to suggest that the head-twitch response to 5-HTP in mice and rats results from a central action of 5-HT (Corne and Pickering, 1967; Corne *et al.*, 1963; Matthews and Smith, 1980; Nakamura and Fukushima, 1978). Among other behavioural models of activation of 5-HT receptors in brain (Grahame-Smith, 1971a, 1971b; Jacobs, 1976; Sloviter *et al.*, 1978), the head-twitch response to 5-HTP has the advantage of being simple in terms of the manipulations and assessments that it involves (Matthews and Smith, 1980).

This study presents an analysis of the effects on the head-twitch response induced by 5-HTP in the rat, of the putative 5-HT receptor blockers metergoline (Beretta, Glässer, Nobili and Silvestri, 1965), methysergide (Fanchamps, Doepfner, Weidmann and Cerletti, 1960), 2-bromo-LSD (Cerletti and Doepfner, 1958), mianserin (Vargaftig, Coignet, De Vos, Grysen and Bonta, 1971), pizotifen (Sicuteri,

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Franchi, Del Bianco and Anselmi, 1966), cyproheptadine (Stone, Wenger, Ludden, Stavorski and Ross, 1961), metitepine (Pelz, Jirkowski, Adlerová, Metyšová and Protiva, 1968) and cinanserin (Rubin, Piala, Burke and Craver, 1964). Also included in this study was pirenperone, a compound which was originally identified (Colpaert *et al.*, 1982) as an LSD-antagonist in the rat.

METHODS

Animals

Male Wistar strain rats, weighing 300 ± 30 g, were used throughout. For observation, the animals were placed in perspex cages measuring 19 × 20 × 16 cm and having a wire mesh floor. The experiments were carried out under conditions of controlled temperature (21 ± 1°C) and humidity (r.h.: 65 ± 5%). Animals were used only once.

Head-twitch response

The head-twitch response that was observed in the present experiments was morphologically similar to the behaviour that has been described as head-twitch (Corne *et al.*, 1963) or head-shake (Matthews and Smith, 1980). It consisted of a characteristic rapid movement of the head with little or no involvement of the trunk. The response was identified and counted by an observer who was unaware of the treatment conditions.

5-Hydroxytryptophan: dose–effect relationship

Rats ($n = 7$ per group) were injected intraperitoneally (i.p.) with either saline or 40, 80, 160, 320 mg/kg of 5-HTP. Head-twitches were counted from 10 until 90 min after the injection.

5-Hydroxytryptophan: time–effect relationship

Nine rats were injected intraperitoneally with 320 mg/kg of 5-HTP and head-twitches were counted per 5 min episode from 0 until 180 min after injection.

5-Hydroxytryptophan assay: control data

The experiments on the effects of putative 5-HT antagonists in the 5-HTP assay involved two control groups. A first group ($n = 139$) consisted of animals that were given 320 mg/kg of 5-HTP (i.p.), and that were injected subcutaneously (s.c.) with saline 10 min later. The second group ($n = 28$) consisted of animals that received an intraperitoneal injection of saline followed 10 min later by a subcutaneous injection of saline. As in drug-treated animals, head-twitches in the two control groups were counted during the 20-min episode commencing 70 min after the intraperitoneal injection (of 5-HTP or saline).

5-Hydroxytryptophan assay: effects of putative 5-HT antagonists

The putative 5-HT antagonists studied were: the ergoline derivatives metergoline, methysergide maleate and 2-bromo-LSD, the cinnamyl derivative cinanserin hydrochloride, cyproheptadine hydrochloride and the cyproheptadine-like tricyclic compounds metitepine maleate, mianserin hydrochloride and pizotifen maleate. The LSD-antagonist pirenperone was also included. The compounds were given subcutaneously 10 min after an intraperitoneal challenge of 320 mg/kg of 5-HTP. Seven rats were used per dose; additional animals were tested to obtain $n = 7$ per dose in those cases where rats died prior to the end of the 20 min observation period. Drug-treated and control animals were run concurrently in experiments involving 6 rats each.

Agonist effects of putative 5-HT antagonists

To match the conditions of the previous experiment, rats were injected subcutaneously with either mianserin, methysergide, pizotifen, cyproheptadine ($n = 7$ per dose) or saline ($n = 22$). Head-twitches were counted during the 20-min episode commencing 60 min after the injection.

Drugs and doses

Doses (see Results section) were selected from the geometric series..., 0.01, 0.04,..., 10, 40,... mg/kg, and refer to the base or salt as indicated above. The volume of intraperitoneal or subcutaneous injections was 1 ml/100 g body weight, except for the 320 mg/kg dose of 5-HTP which was given in 2 ml/100 g. The latter volume was also used with saline where saline injections were to control for the 320 mg/kg dose of 5-HTP.

RESULTS

5-Hydroxytryptophan: dose–effect relationship

An average of 2.5 (+1.5) twitches were observed between 10 and 90 min after saline; these twitches typically occurred soon after the intraperitoneal injection, and few or no responses were observed later than 30 min after saline. 5-Hydroxytryptophan produced a dose-dependent increase in the number of twitches (Fig. 1). This increase was not reliable (Mann–Whitney U-test) at 40 mg/kg (one-tailed $P > 0.05$), but did reach statistical significance at 80 ($P < 0.05$), 160 ($P < 0.01$) and 320 mg/kg ($P < 0.001$).

5-Hydroxytryptophan: time–effect relationship

The onset of the head-twitch response induced by 320 mg/kg of 5-HTP occurred between about the 11th and the 25th min (Fig. 2). Thereafter, the frequency of the response increased to reach a peak of nearly 16 responses/5 min between 70 and 90 min after the injection of 5-HTP. A progressive decline was observed at later times, and the average response frequency fell to 0.5/5 min at 176 to 180 min after administration of 5-HTP.

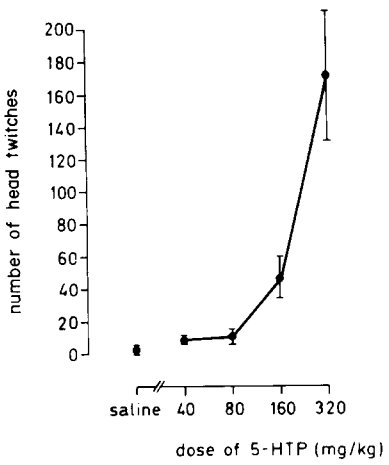

Fig. 1. Head-twitch response induced by 5-HTP in the rat. The number of twitches were counted from 15 until 90 min after the intraperitonal injection of saline or one of several doses of 5-HTP. Data are expressed as the mean (± 1 SEM) of 7 animals per treatment condition.

On the basis of these data (Figs 1 and 2) antagonist drug effects were assayed during the 20-min observation period commencing 70 min after 320 mg/kg of 5-HTP.

5-Hydroxytryptophan assay: control data

Of the 139 control rats that were given 5-HTP (320 mg/kg, i.p., 2 ml/100 g body weight) followed by saline (10 min later; s.c.; 1 ml/100 g body weight), two failed to show any twitch during the 20-min observation period commencing 70 min after injection of 5-HTP. About 80% of these 139 control animals were distributed in an extremely monotonous manner within the response interval ranging from 0 to 80 twitches (Fig. 3). The average count amounted to 54.3 twitches and variability was relatively large (SD: 38.7).

None of the 28 control animals that were given saline (i.p.; 2 ml/100 g body weight) followed by saline (10 min later; s.c.; 1 ml/100 g body weight) showed any twitch.

The frequency distribution of head-twitches in the 5-HTP/saline controls (Fig. 3) and the absence of any twitches in the saline/saline control group indicates that a complete antagonist drug effect to 320 mg/kg 5-HTP in the conditions used requires the drug to reduce the number of twitches to 0.

5-Hydroxytryptophan assay: effects of putative 5-HT antagonists

All eight putative 5-HT antagonists, and the LSD antagonist pirenperone, reduced the number of head-twitches induced by 320 mg/kg of 5-HTP (Figs 4 and 5). Comparison of experimental outcome with the 5-HTP/saline control population by means of the Kolmogorov–Smirnov test indicated significant ($P < 0.05$) drug effects with 0.01–0.63 mg/kg of pirenperone, 0.63–10 mg/kg of 2-bromo-LSD,

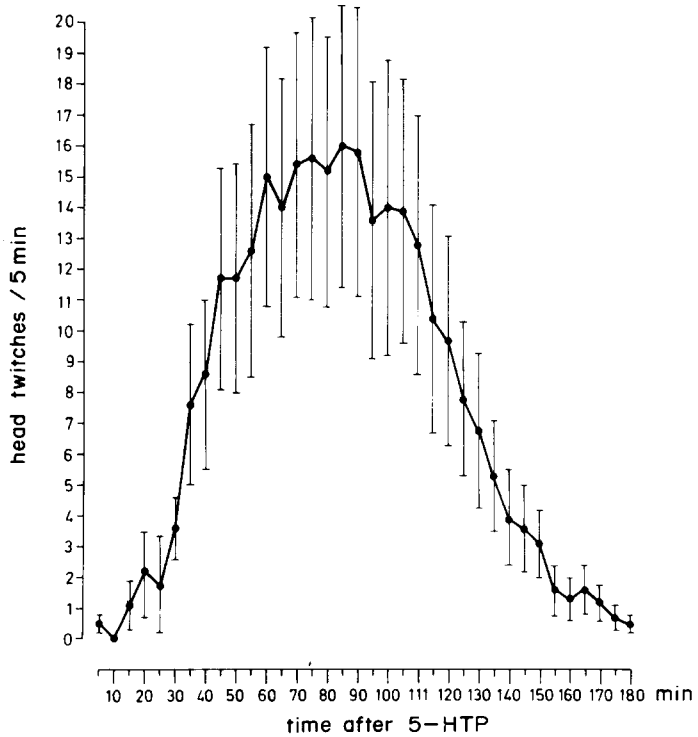

Fig. 2. Time course of the head-twitch response to 320 mg/kg 5-HTP in the rat. The number of shakes were counted in 5-min periods between 0 and 180 min after the intraperitoneal injection of 5-HTP. Data are expressed as the mean (±SEM) of 9 animals.

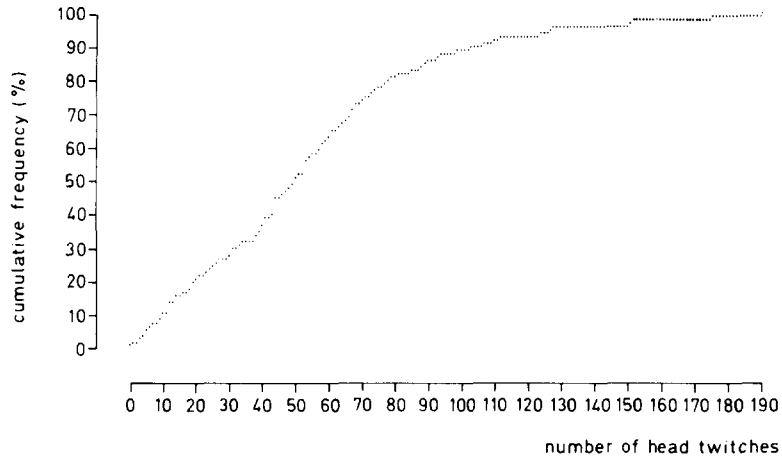

Fig. 3. Cumulative frequency distribution of head-twitches. One hundred and thirtynine control rats were given an intraperitoneal injection of 320 mg/kg of 5-HTP, followed 10 min later by a subcutaneous saline injection. Head-twitches were counted during the 20-min episode commencing 70 min after 5-HTP administration.

0.04–40 mg/kg of mianserin, 0.16–40 mg/kg of methysergide, 0.16–10 mg/kg of metergoline, 0.16–2.5 mg/kg of metitepine, 0.16–40 mg/kg of pizotifen, 0.16–40 mg/kg of cyproheptadine, and with 2.5–160 mg/kg of cinanserin. Some inconsistency was observed with cinanserin; it produced significant inhibition at 0.04 but not at 0.16 mg/kg.

In an effort to fit the data for each drug by simple

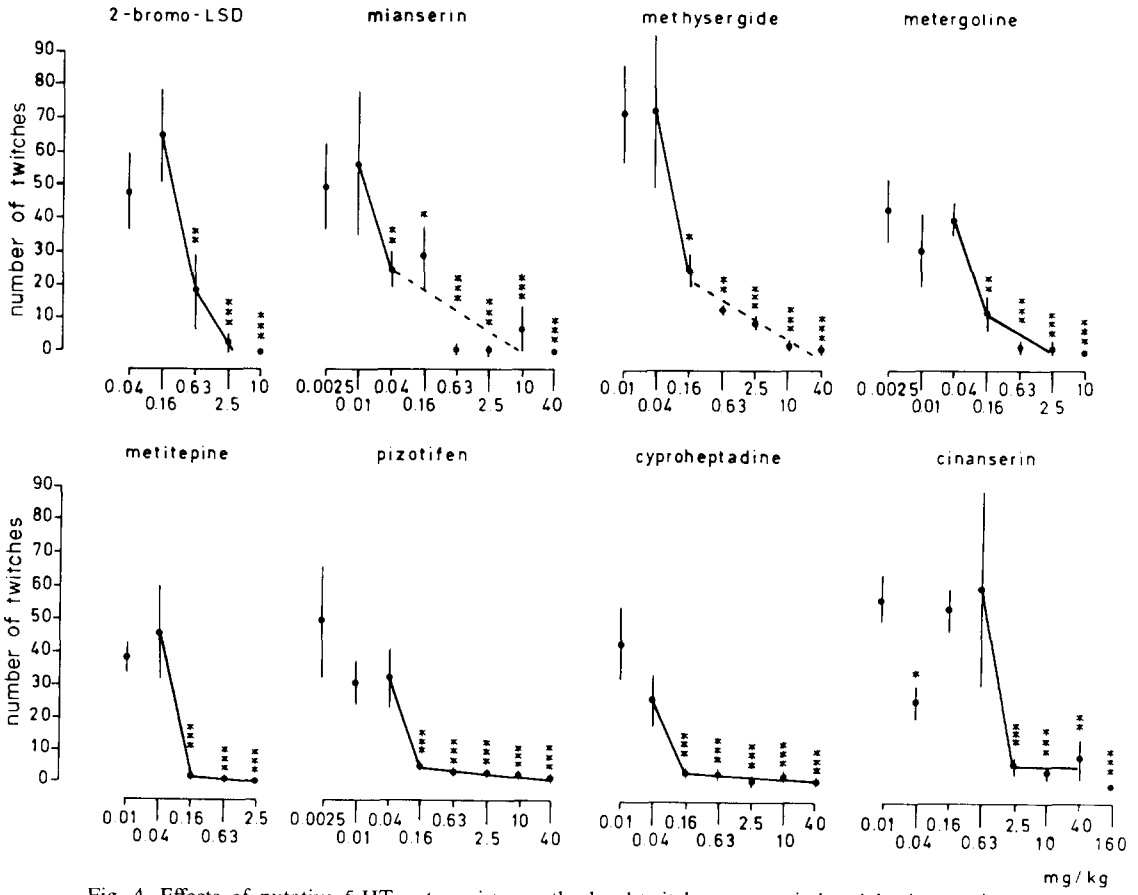

Fig. 4. Effects of putative 5-HT antagonists on the head-twitch response induced by intraperitoneal injections of 320 mg/kg of 5-HTP in the rat. Asterisks indicate two-tailed P (Kolmogorov–Smirnov test) to be * < 0.05, ** < 0.01, or *** < 0.005, respectively, for the difference with saline control. Data are expressed as the mean ± 1 SEM of 7 animals. See text for details.

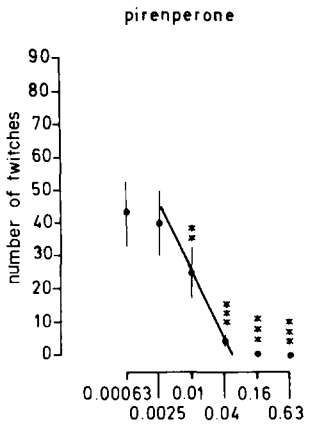

Fig. 5. Effects of pirenperone on the head-twitch response induced by intraperitoneal injections of 320 mg/kg of 5-HTP in the rat. See legend to Fig. 4.

descriptive terms, one or, where necessary, two linear functions were defined. A single linear function provided an adequate fit (χ^2-test; $P > 0.05$) of the data points of 0.0025 to 0.04 mg/kg of pirenperone, the parameter b of the regression equation $y = a + bx$ being -30. With none of the other agents did a single regression line provide a sufficient fit (χ^2-test; $P < 0.05$) of the entire dose–effect relationship; but inspection of the data in Fig. 4 suggested that two consecutive linear functions could offer a simple description of the data. Firstly, the regression equation was computed for those data points which demonstrated significant ($P < 0.05$) inhibition and averaged 1 or more twitches. The linear function so obtained provided a sufficient fit (χ^2-test; $P > 0.05$) for those data points with all antagonists, except with mianserin and methysergide ($P < 0.05$; hence the broken lines in Fig. 4). The antagonists in Fig. 4 appear in their rank order of the slope of this function. Secondly, a straight line was drawn connecting the data point at the largest inactive dose of the drug to that at its smallest active dose.

The dose–effect relationship which so emerges from these linear approximations of the data appears to consist of a single linear function with pirenperone (Fig. 5), but was more complex with the eight putative 5-HT antagonists (Fig. 4). The following general features characterize the dose–effect relationship of these agents in inhibiting the head-twitch response induced by 320 mg/kg of 5-HTP in the rat. The relationship can be said to be biphasic; the initial phase corresponded to a level of effect ranging from no significant inhibition to a point where the response appeared to be inhibited by at least 50%. This first phase occurred with fairly small doses. It had a relatively steep slope (parameter b) which did not differ greatly between the agents being examined, the largest difference being only 2.4-fold (Table 1). The second phase corresponded to a range of effect from $\geqslant 50\%$ to $\leqslant 100\%$ inhibition of the response. With metitepine, pizotifen, cyproheptadine and cinanserin, the mean number of twitches at this point varied from 0 to only 8. The second phase often extended over a broad range of doses, and the slope of this phase differed up to 30-fold between the drugs being examined. In fact, virtually no progression of effect occurred in this phase with metitepine, pizotifen, cyproheptadine and cinanserin (Table 1, Fig. 4). The slope of the second phase of all putative 5-HT antagonists was shallower than that of the first, the differences between the two phases being significant (Wilcoxon test; $P < 0.01$).

At least two general methods are available to estimate the relative potencies of antagonists from the present data. Firstly, the dose producing 50% inhibition (ID_{50}) was found by interpolating the data points above and below 27.1 counts (i.e. half the mean number of head-twitch responses in the 5-HTP/saline control group). Secondly, the ED_{50} was obtained from probit analysis (Finney, 1971) and represents the dose at which complete inhibition was produced in 50% of the animals tested. Complete inhibition was defined as 0 twitches on the basis of

Table 1. Characteristics of drug inhibition of the head-twitch response induced by intraperitoneal injection of 320 mg/kg of 5-HTP in the rat

Drug	Slope I	Slope II	ID_{50}	[Rank]	ED_{50}	[Rank]	ID_{50}
Pirenperone	—	30.0	0.0085	[1]	0.052(0.030–0.090)	[1]	6
2-Bromo-LSD	79.0	25.1	0.47	[8]	2.15 (1.25–3.71)	[5]	5
Mianserin	53.1	10.7	0.036	[2.5]	0.98(0.57–1.69)	[4]	27
Methysergide	79.7	9.8	0.15	[7]	12.1(7.0–20.9)	[7]	81
Metergoline	46.5	9.2	0.076	[6]	0.40(0.23–0.69)	[3]	5
Metitepine	73.1	1.7	0.073	[5]	0.12(0.071–0.21)	[2]	2
Pizotifen	44.8	1.5	0.052	[4]	10.4 (6.0–17.9)	[6]	200
Cyproheptadine	36.5	1.0	0.036	[2.5]	>40	[9]	>1111
Cinanserin	88.5	−0.8	1.50	[9]	19.4(11.3–33.5)	[8]	13

Slope refers to $-b$; b is the parameter b in the equation $y = a + bx$ which fits the first (**I**) and the second (**II**) linear phase of the biphasic dose–response curves depicted in Fig. 4. ID_{50} is the dose (in mg/kg) which inhibited the response by 50%. ED_{50} is the dose (in mg/kg; and 95% confidence limits) which produced complete inhibition of the response in 50% of the animals tested. Also given are the rank order of potencies according to ID_{50} and ED_{50}, as well as the ratio of ED_{50} to ID_{50}.

Table 2. Agonist effects of putative 5-HT antagonists and of pirenperone

Compound	Dose (mg/kg)	N/n	Compound	Dose (mg/kg)	N/n
Saline	—	0/22	Cyproheptadine	0.63	1/7
Mianserin	0.16	0/7		2.5	1/2
	0.63	2/7		10	2/7
	2.5	2/7		40	4/7
	10	0/7	Pirenperone	0.01	0/7
	40	0/7		0.04	0/7
Methysergide	0.63	0/7		0.16	0/7
	10	1/7			
	40	0/7			
Pizotifen	0.16	0/7			
	0.63	2/7			
	2.5	2/7			
	10	1/7			
	40	0/7			

N = number of rats in which more than 1 twitch response was observed during the 20-min observation period commencing 60 min after subcutaneous injection.

the 5-HTP/saline and the saline/saline control data described above. Note that the second method, unlike the first, is binary, or nominal, in nature.

The ID_{50} and ED_{50} values are listed in Table 1. The ID_{50} values revealed drug activity in small doses with most of the agents tested, pirenperone being the most potent. Pirenperone was also most potent according to the ED_{50} index, while cyproheptadine failed to produce complete inhibition in $\geqslant 50\%$ of the animals tested as doses up to 40 mg/kg. Relative potencies varied greatly between the two indices and the potency ranks failed to correlate with any reliable degree (Spearman rank correlation coefficient: $r_s = 0.36$; $P > 0.05$). The ratio of the ED_{50} to the ID_{50} provides some estimate of the rate at which the effect of the drug progressed from 50% to complete inhibition. This ratio was 2 with metitepine, a drug exerting relatively strong neuroleptic activity (Pelz *et al.*, 1968), varied from 5 to 13 with pirenperone, 2-bromo-LSD, metergoline and cinanserin, and was $\geqslant 27$ with mianserin, methysergide, pizotifen and cyproheptadine.

Agonist effects of putative 5-HT antagonists

Following subcutaneous injection of saline, 1 out of 22 (i.e. less than 5%) control rats showed one twitch response during the 20-min observation period commencing 60 min after treatment. Results obtained after drug treatment are therefore expressed (Table 2) as the number of subjects (out of $n = 7$ per dose) showing a number of twitches greater than 1.

Significant ($P < 0.05$) drug responses occurred with each of the 4 putative 5-HT antagonists, but not with pirenperone (Table 2). The absolute number of twitches was at all times small (40 mg/kg cyproheptadine; $\bar{x} = 2.4$; SEM = 0.8) and failed to increase monotonically with mianserin, methysergide and pizotifen. However, the larger doses (10 and 40 mg/kg) of the four agents typically produced general behaviourally depressant effects which may have masked the twitch response.

DISCUSSION

The present data indicate that intraperitoneal injections of 5-HTP in the rat produced head-twitch responses in a dose-related manner (Fig. 1). The time of peak effect of 320 mg/kg of 5-HTP occurred within the 20-min period commencing 70 min after the injection (Fig. 2). At this point, 5-HTP produced an average of about 54 head-twitches, but the number of responses varied considerably between subjects (Fig. 3).

The present experiments confirm earlier data (e.g. Corne *et al.*, 1963; Matthews and Smith, 1980) that putative 5-HT antagonists inhibit the head-twitch response to 5-HTP in rodents. They also specify, however, that this inhibition does not appear as a simple, monophasic, progression of effect. The dose–response curve for these agents in antagonizing the head-twitch response to 320 mg/kg of 5-HTP in the rat accommodated a biphasic curve (Fig. 4). The first phase consisted of a steep reduction of response frequency from 54 to less than half this number; the slope of this phase varied only $\leqslant 2.4$-fold between the agents being examined. The second phase typically consisted of a further, but shallower reduction of response frequency; the slope of this phase varied up to 30-fold between the agents, and little or no progression of effect (parameter $b \simeq 1.0$) occurred at this point with metitepine, pizotifen, cyproheptadine and cinanserin (Fig. 4; Table 1). Only pirenperone produced a monophasic inhibition of the head-twitch response (Fig. 5).

The implication of this data for the use of the head-twitch response to 5-HTP as a pharmacological assay (Matthews and Smith, 1980) is that the rank order of antagonist drug potency is, in fact, method-dependent (Table 1). That is, expressing potency of the drug by the dose producing 50% inhibition of the number of head-twitches (a quantitative method) yields a rank order of potency which even fails to correlate with that of the ED_{50} in producing complete inhibition of the response (a quantal method). It is not immediately apparent which of these two methods, if any, offers a more valid measure of 5-HTP-antagonist effects. One source of discrepancy, however, is that the quantitative and the quantal estimates yielded doses that typically occurred within the first and second phase, respectively.

Peroutka, Lebovitz and Snyder (1981) have used the head-twitch response to 5-HTP as a behavioural assay in an effort to validate the 5-HT_2 binding site (Leysen, Niemegeers, Tollenaere and Laduron, 1978; Peroutka and Snyder, 1979) as a 5-HT receptor. Using a quantitative method of analysis it was found that binding to the 5-HT_2 site correlated with the potency of the drug in reducing 5-HTP-induced head-twitches. The finding that pirenperone antagonized the response (Fig. 5) supports the suggestion, then, that the 5-HTP-induced head-twitch involves a 5-HT_2 receptor. Pirenperone binds to the 5-HT_2 site, but has no affinity for the 5-HT_1 site (Colpaert and leysen, 1981; Leysen, Niemegeers, Van Nueten and Laduron, 1982).

The biphasic shape of the dose–effect curve for putative 5-HT antagonists in the present experiments (Fig. 4) resembles, at least superficially, the curve for these agents in antagonizing the cuing properties of LSD in the rat (Colpaert *et al.*, 1982). The second, shallow phase in the latter study was interpreted to reflect the paradoxical agonist activity that these agents may produce at doses in excess of those at which antagonist effects become apparent. This suggestion arose from findings (Colpaert, Niemegeers and Janssen, 1979; Colpaert *et al.*, 1982) that these agents in part mimick, in addition to partly antagonizing, the LSD cue in the rat. A similar interpretation can be made of the present data; while the number of head-twitches produced by any 5-HT antagonist was invariably small, significant 5-HTP-like agonist effects did occur (Table 2). It seems, therefore, that the occurrence of partial agonist activity accounts for the failure of the first phase to extend to larger doses, thus resulting in a second, shallow phase. At any rate, the occurrence of any twitches at the larger pretreatment doses of these compounds (Fig. 4) is surprising in view of the behavioural toxicity which is apparent at these doses (Colpaert *et al.*, 1982) and must be taken to reflect a relative inability to fully antagonize the effects of 5-HTP.

The biphasic shape of the curve for putative 5-HT antagonists in the present (Fig. 4) and earlier experiments (Colpaert *et al.*, 1982) is difficult to understand at the level of drug–receptor interaction. A simple, possibly competitive, interaction at 5-HT_2 sites can conceivably account for the first, steep phase in the antagonism of the LSD cue or the head-twitch response to 5-HTP. The second phase, however, requires that an additional mechanism becomes operative. It is of interest, then, that the sole pure antagonist in these experiments, i.e. pirenperone, differs from the putative 5-HT antagonists in that it fails to bind to 5-HT_1 sites (Colpaert and Leysen, 1981). The fact that all other putative antagonists tested here do bind to 5-HT_1 receptors (Leysen, Awouters, Kennis, Laduron, Vandenberk and Janssen, 1981) may therefore relate, in some unspecified manner, to the occurrence of a second, slow phase in the antagonist dose–response curve.

In conclusion, the present data indicate that a number of putative 5-HT antagonists reduce the frequency of the head-twitch response to 5-HTP in the rat. The antagonist dose–effect curve of these agents was typically biphasic; it consisted of a first, steep phase and a second, shallow phase. Pirenperone antagonized 5-HTP according to a simple, monophasic dose–effect curve. The antagonist effects of pirenpirone, and the first, steep phase of the purported 5-HT antagonists, may reflect antagonist drug activity at 5-HT_2 receptors. The second, shallow phase may result from paradoxical agonist activity; the molecular mechanism of these agonist effects is only poorly understood. Unlike earlier 5-HT antagonists, pirenperone acted as a pure antagonist, rather than exerting complex, mixed agonist/antagonist effects.

Acknowledgement—We gratefully acknowledge Ms L. Raeymaekers for expert technical assistance. This work was supported in part by a grant from IWONL.

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