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Behavioural Actions of the Serotonergic Anxiolytic Indorenate

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FERNÁNDEZ-GUASTI, A., A. ESCALANTE, E. HONG AND A. AGMO. *Behavioural actions of the serotonergic anxiolytic indorenate*. PHARMACOL BIOCHEM BEHAV **37**(1) 83–88, 1990.—Several recent studies have shown that the 5-HT_{1A} agonist indorenate possesses antianxiety properties. In the present study we report on other behavioural actions of this drug. Indorenate (31.6 mg/kg) induced flat body posture, forepaw treading and hind limb abduction, behavioural characteristics of the serotonin syndrome. After indorenate injection these same behaviours were observed in animals pretreated with p-chlorophenylalanine (400 mg/kg × 3 days), suggesting that the action of this compound is not mediated via serotonin release. The beta-5-HT_1 blockers, (−) pindolol (2 mg/kg) or (−) alprenolol (5 mg/kg), did not prevent the actions of indorenate on the serotonin syndrome. Indorenate (10 mg/kg) stimulated the masculine sexual behaviour by reducing the number of intromissions preceding ejaculation. Higher doses (17.8 mg/kg) cause a complete inhibition of sexual behaviour. (−) Pindolol (2 mg/kg) or (−) alprenolol (5 mg/kg) did not antagonize the facilitatory actions of indorenate on male sexual behaviour. A high dose of indorenate (31.6 mg/kg) resulted in an impairment of the motor coordination as tested in a treadmill apparatus. These data reveal that indorenate possesses, in addition to its antianxiety effects, other behavioural characteristics that, however, appear at higher dose levels.

Indorenate "Serotonin syndrome" Masculine sexual behaviour Motor coordination pCPA (−) Pindolol (−) Alprenolol 5-HT_1 agonist

SEVERAL data derived from receptor-binding (10,26), neurochemical (23) and antihypertensive (22, 24, 25) studies have suggested that indorenate, a new serotonergic analogue (30), is an agonist at the serotonin 1A binding site (5-HT_{1A}).

Recently, we reported on the anxiolytic actions of indorenate (13–15). From these data we proposed that indorenate produces its antianxiety effects via stimulating the 5-HT_{1A} receptors (14). The 5-HT_{1A} agonists may produce, in addition to their anxiolytic properties [cf. (16,17)], the induction of some characteristics of the "serotonin syndrome": flat body posture, forepaw treading and hind limb abduction (5, 19, 21, 29, 33) and the stimulation of masculine sexual behaviour shown as a reduction in the number of intromissions preceding ejaculation (2, 3, 6, 11, 28).

The purpose of the present study was to analyze if indorenate possesses these behavioural characteristics and whether these actions were mediated via the stimulation of the 5-HT_{1A} receptor. An additional experiment using the serotonin synthesis inhibitor, p-chlorophenylalanine (pCPA), was included in order to determine if the actions of indorenate were mediated via a serotonin-releasing mechanism.

METHOD

Subjects

Adult male Wistar rats (250–350 g body weight), bred in a local colony, were used in this study. Animals were individually housed in a room with a controlled light:dark cycle (12-hr light: 12-hr dark). All animals had free access to tap water and Purina rat chow.

Drugs

The following drugs were used in this study: quipazine maleate (Miles Laboratories, Elkhart, IN), indorenate (TR 3369, Miles Laboratories, Inc., CINVESTAV, IPN, México City, México), (−) pindolol (Sandoz, Basel, Switzerland), (−) alprenolol (Hässle AB, Mölndal, Sweden) and p-chlorophenylalanine (Sigma Chemicals, St. Louis, MO). All drugs, except pCPA, were dissolved in physiological saline and injected IP in a volume of 2.0 ml/kg. PCPA was suspended in methyl cellulose and IP injected in a volume of 4.0 ml/kg. In all experiments indorenate was injected 90 min before behavioural observations. This latency was chosen on the basis of previous neurochemical and behavioural data showing maximum effects at this time (data not shown). The interval for the other compounds used in this study is stated under each experimental procedure.

Steroids

The steroids used were testosterone (T), oestradiol benzoate

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(OB) and progesterone (P). T and OB were purchased from Sigma Chemicals, St. Louis, MO and P was purchased from Aldrich, Milwaukee, WI. OB and P were dissolved in sesame oil and SC injected in a volume of 0.4 ml/kg.

Experiment 1. Effect of Various Doses of Indorenate on the Serotonin Syndrome

Animals were placed in a clear plastic cage measuring 26 × 30 × 13 cm 10 min before the beginning of the observations. The observations were made during 28 min, 10 min after quipazine (50 mg/kg) or 90 min after indorenate (10–31.6 mg/kg) or saline (2 ml/kg) administration. Sequential observations were made during one min, leaving a seven-min interval between each recording period (the observer was "blind" to the drug treatment).

The parameters recorded and the way of registering them were as follows: head weaving, forepaw treading and straub tail (0 = absent; 1 = present once; 2 = present several times; 3 = present frequently; 4 = present continuously); hind limb abduction, tremor and flat body posture (0 = absent; 1 = perceptible; 2 = weak; 3 = medium; 4 = maximal) and head/body shakes (frequency per scoring period). At the end of the observations a behavioural score was calculated for each treatment (4 scoring periods, maximum score per behaviour = 16 except for head/body shakes). The statistical analysis was made between the indorenate-treated groups and the saline- or quipazine-injected animals using the Mann-Whitney U-test (31).

Experiment 2. Effect of Treatment With (−) Alprenolol, (−) Pindolol or pCPA on the Serotonin Syndrome Induced by Indorenate

The serotonin syndrome was recorded according to the method described in Experiment 1. In the first series of experiments, thirty-five animals were used. Half the animals were injected daily with pCPA (400 mg/kg) or methyl cellulose (4 ml/kg) during three consecutive days beginning 72 hr before the observations. This treatment with pCPA has been previously described as an effective manipulation to specifically inhibit 5-HT synthesis (27). Twenty-four hr after the last injection the animals were divided into two groups receiving saline (2 ml/kg) or indorenate (31.6 mg/kg); 90 min after this injection the serotonin syndrome was recorded. The statistical analysis was made between the pCPA and the methyl cellulose-treated groups and between the indorenate-injected groups using the Mann-Whitney U-test (31).

In other series of experiments, (−) alprenolol (5 mg/kg) or (−) pindolol (2 mg/kg) were administered either simultaneously (90 min before the observations) or 60 min after indorenate (31.6 mg/kg) injection and the serotonin syndrome registered. All groups in these series were compared versus the indorenate- (31.6 mg/kg) plus saline- (2 ml/kg) treated group using the Mann-Whitney U-test (31).

Experiment 3. Effect of Various Doses of Indorenate on Masculine Sexual Behaviour

All animals used in this experiment were kept in a room under a reversed light: dark cycle (12-hr light: 12-hr dark, lights on at 2000 hr). The males used in this experiment received three mating tests of 30 min duration. Those animals that achieved one ejaculation in at least one of the tests were castrated under methohexital (40 mg/kg) anesthesia. Within one hour after castration, all animals were SC implanted with a 20 mm long T-filled capsule (0.062 in, internal diametre; 0.125 in, external diametre, Dow Corning). One week before drug treatment, a test for sexual behaviour was performed and those males showing an ejaculation latency longer than 15 min were eliminated. Stimulus female Wistar rats (200–300 g body weight) were used for the mating tests. All females were ovariectomized under methohexital (40 mg/kg) anesthesia three weeks before sexual behaviour observations. Female rats were brought into sexual receptivity by the sequential administration of oestradiol benzoate (25 μg/rat, 48 hr) followed by progesterone (1.0 mg/rat, 4 hr).

Sexual behaviour observations were begun six hours after the onset of darkness. The male rat was placed in the observation cage (40 × 60 × 30 cm) five min before the receptive female rat was introduced. During the test the following behavioural parameters were recorded:

1. Intromission latency: time from the introduction of the female until the first mount with vaginal penetration.
2. Mounts and intromissions: number of mounts and intromissions preceding ejaculation.
3. Ejaculation latency: time from the first intromission until ejaculation.
4. Postejaculatory interval: time from the ejaculation until the first intromission of the second series of copulation.

Mounts, intromissions and ejaculation were recognized because of their particular behavioural characteristics. Indorenate (3.1–17.8 mg/kg) or saline (2 ml/kg) were administered according to a balanced latin square design in such a way that each animal received saline and all the dosages of the drug. The effect of the drug test on the masculine sexual behaviour was evaluated with the Friedman two-way ANOVA test followed by the Wilcoxon matched-pairs signed-ranks test (31). The proportion of animals showing mounts, intromissions and ejaculation was evaluated using the Cochran Q-test followed by the binomial test (31).

Experiment 4. Effect of (−) Alprenolol or (−) Pindolol on the Actions of Indorenate on Masculine Sexual Behaviour

In these series of experiments two balanced latin square designs involving (−) alprenolol (5 mg/kg) or (−) pindolol (2 mg/kg), together with indorenate (10 mg/kg) were used. Indorenate and each of the antagonists were simultaneously injected 90 min before the observations. The parameters registered and the way of analyzing the data were the same as previously described.

Experiment 5. Effect of Various Doses of Indorenate and Indorenate Combined With (−) Alprenolol or (−) Pindolol on a Motor Coordination Test (Treadmill Test)

For this experiment a treadmill (rotarod) apparatus was used. The procedure followed has been previously described by Agmo *et al.* (1). Briefly, trained animals were placed upon a cylinder (diametre, 7 cm) rotating at a speed of 11 rpm. After saline (2 ml/kg), indorenate (10–31.6 mg/kg) or the combined treatment of indorenate (10 mg/kg) plus (−) alprenolol (5 mg/kg) or plus (−) pindolol (2 mg/kg), the number of falls during a 5-min period was counted. After a fall the animal was replaced on the cylinder. Each experimental group was subjected to a predrug test 24 hr before the treatment. All treatments were administered 90 min before the tests. The data were statistically analyzed using the Wilcoxon matched-pairs signed-ranks test (31).

RESULTS

Experiment 1. Effect of Various Doses of Indorenate on the Serotonin Syndrome

Administration of quipazine (50 mg/kg) resulted in the appearance of all the components of the serotonin syndrome in all

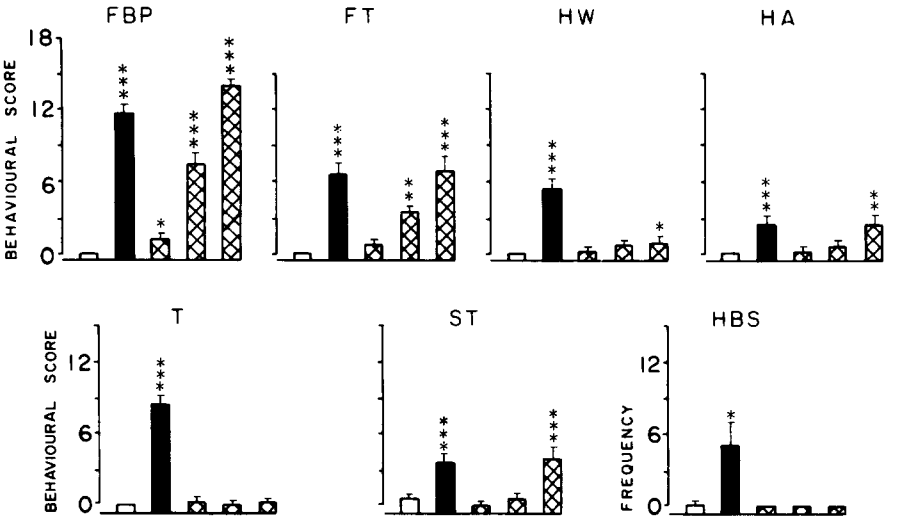

FIG. 1. Effect of indorenate (10–31.6 mg/kg) on the serotonin syndrome. Open bars: saline control; full bars: quipazine (50 mg/kg) control; dashed bars: ascendent doses of indorenate (10, 17.8 and 31.6 mg/kg). FBP: flat body posture; FT: forepaw treading; HW: head weaving; HA: hind limb abduction; T: tremor; ST: straub tail; and HBSH: head/body shakes.

subjects, while no component of this syndrome was observed after saline (Fig. 1). Indorenate at the 10.0 or 17.8 mg/kg dose level induced only a weak stimulation of some of the behaviours that characterize this syndrome (flat body posture, forepaw treading and head weaving). A higher dose of indorenate (31.6 mg/kg) resulted in an intense display of some of the components of this syndrome: flat body posture, forepaw treading, hind limb abduction and straub tail. Other components of the "serotonin syndrome," such as tremor and head/body shakes, were completely absent after indorenate administration.

Experiment 2. Effect of (−) Alprenolol, (−) Pindolol or pCPA on the Serotonin Syndrome Induced by Indorenate

As in Experiment 1 the administration of indorenate (31.6 mg/kg) resulted in the appearance of some components of the serotonin syndrome such as flat body posture, forepaw treading, hind limb abduction and straub tail. Saline injection did not induce any component of the serotonin syndrome in pCPA-, (−) alprenolol- or (−) pindolol-pretreated rats (data not shown). Furthermore, in pCPA-pretreated animals, indorenate (31.6 mg/kg) injection produced the serotonin syndrome in an extent similar to that methyl cellulose-pretreated animals. A slight increase in the hind limb abduction component was observed in pCPA-pretreated rats, however, the comparison of the other components did not reveal statistically significant differences (Fig. 2). The systemic injection of (−) alprenolol (5 mg/kg) or (−) pindolol (2 mg/kg) did not produce any component of the serotonin syndrome (data not shown). Furthermore, the treatment with these antagonists did not prevent the induction of the serotonin syndrome by indorenate

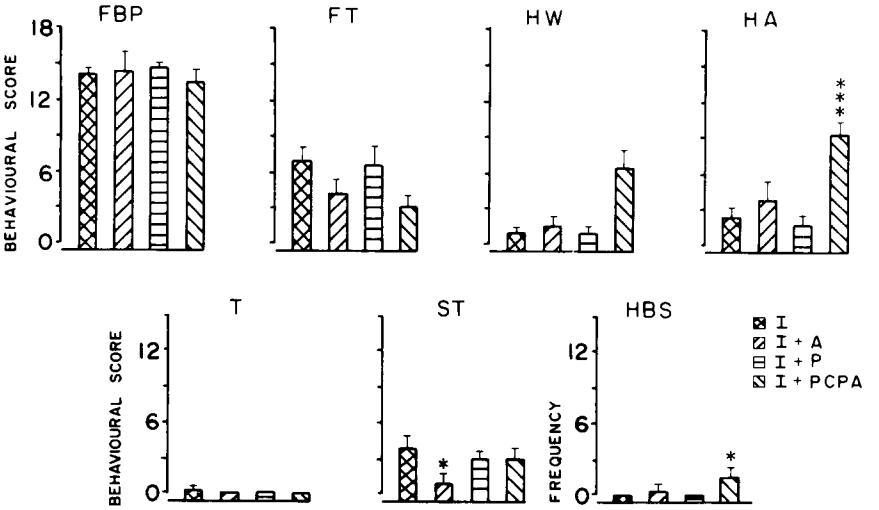

FIG. 2. Effect of indorenate (31.6 mg/kg) alone or in combination either with (−) alprenolol (5 mg/kg); (−) pindolol (2 mg/kg) or pCPA (400 mg/kg × 3 days) on the serotonin syndrome. Abbreviations as in Fig. 1.

TABLE 1

PROPORTION OF ANIMALS SHOWING MOUNTS, INTROMISSIONS AND EJACULATION AFTER THE ADMINISTRATION OF INDORENATE (3.1–17.8 mg/kg) OR SALINE (2 mg/kg) DURING A 30-MINUTE MATING TEST

Treatment (mg/kg)	Mount (%)	Intromission (%)	Ejaculation (%)
Saline	100 (9/9)	100 (9/9)	100 (9/9)
Indorenate (3.1)	100 (9/9)	100 (9/9)	100 (9/9)
Indorenate (5.7)	100 (9/9)	100 (9/9)	100 (9/9)
Indorenate (10.0)	100 (9/9)	100 (9/9)	100 (9/9)
Indorenate (17.8)	11[b] (1/9)	0[b] (0/9)	0[b] (0/9)
Q[a] =	32.0	36.0	36.0
$p<$	0.001	0.001	0.001

Statistical comparisons were made between saline- and indorenate-treated groups. [a]Cochran Q-test; [b]Binomial test. *$p<0.001$.

(Fig. 2). Finally, the administration of these antagonists, at the same doses, 60 min after indorenate injection, were equally ineffective to block the serotonin syndrome (data not shown).

Experiment 3. Effect of Various Doses of Indorenate on Masculine Sexual Behaviour

Table 2 shows that administration of indorenate (10 mg/kg) resulted in a facilitation of masculine sexual behaviour evidenced as a reduction in the number of intromissions preceding ejaculation. In this experiment, however, this dose of indorenate also induced a small, though statistically significant, prolongation of the postejaculatory interval. A higher dose of indorenate (17.8 mg/kg) caused a drastic inhibition of the copulatory behaviour. Only one of the animals treated with this dose displayed occasional mounts (Table 1). Lower doses of indorenate did not affect the proportion of animals showing sexual behaviour (Table 1).

Experiment 4. Effect of (−) Alprenolol or (−) Pindolol on the Action of Indorenate on Masculine Sexual Behaviour

Table 3 shows the results of these experiments. As previously shown in Experiment 3, the administration of indorenate (10 mg/kg) produced in a reduction in the number of intromissions preceding ejaculation (Table 3). This effect was observed along the two independent experimental series. By contrast, the prolongation of the postejaculatory interval, found in Experiment 3, was not observed in these experiments. The administration of (−) alprenolol (5 mg/kg) (−) pindolol (2 mg/kg) per se did not modify any parameter of sexual behaviour. Furthermore, these compounds, at the doses tested, failed to prevent the reduction in the number of intromissions produced by indorenate administration (Table 3).

TABLE 2

EFFECTS OF INDORENATE (3.1–17.8 mg/kg) ON MASCULINE SEXUAL BEHAVIOUR

Treatment (mg/kg)	IL	NM	NI	EL	PEI
Saline	0.4	7	12	7.7	5.2
Indorenate (3.1)	0.3	9	10	4.8	5.4
Indorenate (5.7)	0.2	8	12	9.5	6.4
Indorenate (10.0)	0.6	8	8†[b]	6.8	6.8*[b]
Indorenate (17.8)	—	—	—	—	—
χ^{2a}	3.36	4.07	10.43	1.8	7.8
$p<$	NS	NS	0.015	NS	0.05

Table shows median values (n = 9). IL: intromission latency; NM: number of mounts; NI: number of intromissions; EL: ejaculation latency; and PEI: postejaculatory interval. [a]Friedman two-way ANOVA test; [b]Wilcoxon matched-pairs signed-ranks test. *$p<0.05$; †$p<0.025$.

TABLE 3

EFFECTS OF (−) ALPRENOLOL (5 mg/kg) AND (−) PINDOLOL (2 mg/kg) IN COMBINATION WITH INDORENATE (10 mg/kg) ON MASCULINE SEXUAL BEHAVIOUR

Treatment (mg/kg)	IL	NM	NI	EL	PEI
Saline	0.9	7	9	6.1	6.6
Indorenate (10.0)	1.0	8	6*[b]	7.3	6.4
(−) Alprenolol (5.0)	0.3	6	8	7.6	5.6
Indorenate (10.0) + (−) Alprenolol (5.0)	1.1	5	6*[b]	7.4	7.4
χ^{2a}	0.91	2.24	8.51	1.40	3.69
$p<$	NS	NS	0.05	NS	NS
Saline	0.6	3	7	5.1	5.7
Indorenate (10.0)	0.3	2	5†[b]	4.7	6.6
(−) pindolol (2.0)	0.2	2	8	7.7	6.2
Indorenate (10.0) + (−) Pindolol (2.0)	0.2	2	6 NS	6.3	6.3
χ^{2a}	0.61	1.21	14.67	6.03	1.93
$p<$	NS	NS	0.01	NS	NS

Table shows median values (−) alprenolol, n = 8; (−) pindolol, n = 9. Abbreviations as in Table 2. [a]Friedman two-way ANOVA test; [b]Wilcoxon matched-pairs signed-ranks test. *$p<0.05$; †$p<0.01$.

Experiment 5. Effect of Various Doses of Indorenate and the Combined Treatment of (−) Alprenolol or (−) Pindolol and Indorenate in a Motor Coordination Test (Treadmill Test)

The motor coordination, as tested in a treadmill test, was not impaired after the injection of 10 or 17.8 mg/kg of indorenate. However, motor deficiencies were observed after the administration of a higher dose (31.6 mg/kg). Thus, the mean ± S.E. number of falls during a 5-min test was 42.2 ± 8.7 after indorenate and 2.0 ± 1.2 after control treatment (Wilcoxon matched-pairs signed-ranks test, $p<0.01$). The combined treatment with indorenate (10 mg/kg) and (−) alprenolol or (−) pindolol did not result in changes in the motor coordination (data not shown).

DISCUSSION

Present data show that a high dose of indorenate (31.6 mg/kg) induces some of the components that characterize the serotonin syndrome such as flat body posture, forepaw treading, hind limb abduction and straub tail. It has been shown that the administration of high doses of the other 5-HT_{1A} agonists, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) (21,34), 5-methoxy dimethyl tryptamine (5-MeODMT) (19, 29, 35), RU 24969 (36) and buspirone (20) produces similar behavioural responses.

In the analysis of the serotonin syndrome, Smith and Peroutka

(33) have shown different profiles within the group of 5-HT_{1A} agonists. Thus, 8-OH-DPAT and 5-MeODMT induce flat body posture, straub tail, forepaw treading, head weaving and hind limb abduction. Conversely, buspirone produces only hind limb abduction, flattened body posture and straub tail, while ipsapirone induces only a slight flat body posture. From these data it was concluded that 8-OH-DPAT and 5-MeODMT may be considered as full agonists. The present results show that indorenate, at higher doses than 8-OH-DPAT and 5-MeODMT, produces the same components, therefore making it possible to propose that indorenate is also a full agonist at least with regard to the 5-HT syndrome. Further experiments involving the interaction between these drugs should be undertaken to fully confirm this idea.

It has been shown that administration of the 5-HT releasers p-chloroamphetamine (pCA) and fenfluramine may induce the serotonin syndrome (9,37). Thus, from the present data it could be argued that indorenate induces the serotonin syndrome via serotonin release. This possibility, however, was excluded by the experiment showing that indorenate produces the syndrome in animals pretreated with a serotonin synthesis inhibitor. Indeed, as previously reported with other 5-HT agonists (19), some of the components of the syndrome appear even more pronounced when indorenate was administered to pCPA-pretreated animals after indorenate. These results indicate that the action of indorenate is direct on postsynaptic 5-HT_{1A} receptors.

Analysis of the blockade of the 5-HT syndrome induced by various 5-HT_1 antagonists reveal several controversies. Thus, some authors (19,29) have been able to block some components of the serotonin syndrome induced either by 5-MeODMT or 5-hydroxytryptophan with 5-HT antagonists such as metergoline, methysergide, methiotepin and (−) propranolol. Conversely, other authors (21,32) have failed to block the 5-HT syndrome induced by 8-OH-DPAT and lisuride after administering nonselective 5-HT antagonists. The present results, showing a lack of antagonism of (−) alprenolol and (−) pindolol on the 5-HT syndrome induced by indorenate, are in line with the latter observations.

Although masculine sexual behaviour cannot be considered as a paradigm for studying putative new serotonergic agonists due to its complex characteristics, it is worth noting that several 5-HT_{1A} agonists including 8-OH-DPAT (2, 3, 28), 5-MeODMT (11), ipsapirone (12,18) and RDS-127 (7,8) share the property of stimulating the copulatory behaviour by reducing the number of intromissions preceding ejaculation. Present data showing that indorenate (10 mg/kg) stimulates masculine sexual behaviour in a similar way to that of other 5-HT_{1A} agonists lead to the conclusion that the number of intromissions preceding ejaculation is regulated by the 5-HT_{1A} receptor type. The present data also show that indorenate at higher doses (17.8 mg/kg) completely inhibits masculine sexual behaviour. This last finding cannot be explained on the basis of a lack of motor capacities, since at this dose, indorenate does not impair the motor coordination as tested in a rotarod test. These data indicate that indorenate induces a dual effect on masculine sexual behaviour, i.e., at lower doses (10 mg/kg) facilitating and at higher doses inhibiting this behaviour. We have recently proposed (12) that the stimulation of the 5-HT_{1B} receptor subtype has an inhibitory action on masculine sexual behaviour. Thus, the inhibitory effect of indorenate on copulation could be due to the ability of this compound to stimulate, in addition, and in a lower extent than the 5-HT_{1A}, the 5-HT_{1B} receptor subtype (26). Further experiments, however, are required to confirm this idea.

It is interesting to note the relationship between the dose of the 5-HT_1 agonist required for facilitating the masculine sexual behaviour and its binding capacity [cf. (26)]. Thus, very low doses of compounds with high affinity for the 5-HT_{1A} recognition site (e.g., 8-OH-DPAT) are required for the stimulation of sexual behaviour. Higher doses of compounds with comparatively lower affinity for the 5-HT_{1A} receptor subtype are needed for the stimulation of the copulatory behaviour. Present data are in line with this observation and further support the idea that the 5-HT_{1A} receptor subtype mediates the number of intromissions preceding ejaculation.

The injection of various 5-HT antagonists have failed to prevent the facilitatory action of 8-OH-DPAT on male sexual behaviour (3). However, it has recently been shown (4) that the administration of (−) pindolol and (−) alprenolol was able to prevent such facilitation. In that experiment, high doses of (−) alprenolol and (−) pindolol, with inhibitory effects per se were used, thereby complicating the interpretation. In the present experiments neither (−) alprenolol nor (−) pindolol, at doses that per se affected the sexual behaviour, antagonize the indorenate action on the number of intromissions preceding ejaculation. Needless to mention, the introduction of selective 5-HT_{1A} antagonists would be required to specify the action of indorenate on sexual behaviour.

Interestingly, indorenate antianxiety effects in mice were effectively blocked by the 5-HT_{1A} antagonists, methiotepin, pindolol and alprenolol (14). Additionally, the indorenate effects on the dog external carotid blood flow were also prevented by these antagonists (24,25). On the basis of these experiments a species differential role of the 5-HT_{1A} antagonists in preventing indorenate actions could be suggested. This idea is at present studied in our laboratory.

It is worth mentioning that present behavioural actions of indorenate, in contrast to its antihypertensive effects (22, 24, 25, 30), and to its antianxiety properties (13–15) occur at relatively high doses (10.0 mg/kg IP, 2.5 and 5.0 mg/kg IP, and 1.0 or 3.0 mg/kg, PO, respectively). This finding suggests that indorenate is more selective for the central receptors regulating the antianxiety processes and the blood pressure mechanisms than for those involved in the behavioural actions observed in this study. As to its antihypertensive properties, this proposal is further supported by recent findings showing that indorenate induces a marked decrease in 5-hydroxyindolacetic acid (5-HIAA) in the brain stem (23), which is involved in the regulation of blood pressure.

In conclusion, the present series of experiments demonstrate that indorenate possesses, in addition to its antianxiety effects, other behavioural characteristics that, however, appear after higher doses.

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