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Behavioural Evidence for Central D-2 Dopamine Receptor Agonistic Effect by Some 2-(Fluorohydroxyphenyl)Ethylamines

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FERRARI, F. AND F. CLAUDI. *Behavioural evidence for central D-2 dopamine receptor agonistic effect by some 2-(fluorohydroxyphenyl)ethylamines.* PHARMACOL BIOCHEM BEHAV **38**(1) 131–134, 1991.—The IP injection of 2-(4-fluoro-3-hydroxyphenyl)ethylamine (FDA 24), N-n-propyl-N-(2-phenylethyl)-2-(3-fluoro-4-hydroxyphenyl)ethylamine (FDA 27F) and N-n-propyl-N-(2-phenylethyl)-2-(4-fluoro-3-hydroxyphenyl)ethylamine (FDA 40) into adult male rats induced the stretching and yawning (SY) syndrome, FDA 24 being the least active. Moreover, FDA 27F and FDA 40 potentiated penile erection (PE) with respect to controls. For both signs (PE and SY), FDA 40 was the most potent of the three compounds. These effects, which are considered typical signs of central D-2-dopamine (DA) receptor stimulation, were dose-related and significantly inhibited by pretreatment with the selective D-2 DA antagonist, sulpiride, but not by domperidone, which does not cross the hematoencephalic barrier. In previous binding studies, FDA 27F and FDA 40 showed high affinity and selectivity for D-2 DA receptors, while FDA 24 had a low affinity for both D-1 and D-2 DA receptors. The present data show that FDA 27F and FDA 40 cross the blood-brain barrier and exert an agonistic effect on the central D-2 DA receptors. These results also provide evidence of the value of PE and SY tests as sensitive tools for the study of DA-neurochemical mechanisms.

Dopamine D-2 receptors Penile erection Stretching and yawning 2-(Fluorohydroxyphenyl)ethylamines

REPEATED episodes of penile erection (PE) and stretching and yawning (SY) are typically produced by all D-2 agonists, and by D-1/D-2 DA agonists when administered to male rats at doses incapable of eliciting a marked degree of stereotyped behaviour (SB) (2). The observation that the selective D-2 DA antagonist, sulpiride (4, 11, 13, 14), is active in antagonizing these syndromes would suggest that D-2 receptor mechanisms are primarily involved (1); thus PE and SY evaluation represents a simple behavioural method for the detection of agonistic activity on D-2 DA receptors (7–9, 12, 16).

The new compounds, FDA 24, FDA 27F and FDA 40 (Fig. 1), have recently been synthesized. They can be regarded as modifications of the DA molecule in which one of the hydroxyl groups of the catechol moiety is replaced by fluorine and the amino group is substituted by n-propyl and 2-phenylethyl groups. Binding studies performed with [^{3}H]SCH 23390 (D-1 selective) and [^{3}H]spiperone (D-2 selective) as radioligands showed that FDA 24 (D-1, $IC_{50}=6.15$ μM; D-2, $IC_{50}=4.64$ μM) has about half the degree of affinity for D-1 and D-2 binding sites that DA does (D-1, $IC_{50}=3.0$ μM; D-2, $IC_{50}=2.2$ μM). On the other hand, FDA 27F and FDA 40, were, respectively, four and ten times more effective than DA in displacing [^{3}H]spiperone and showed high selectivity for D-2 receptors (FDA 27F: D-1 $IC_{50}=38$ μM, D-2 $IC_{50}=0.58$ μM; FDA 40: D-1 $IC_{50}=10.9$ μM, D-2 $IC_{50}=0.25$ μM) (3).

The present work was designed to examine the effects of the new compounds on PE and SY and to find out whether a behavioural study would support the biochemical findings. Moreover, the experiments could determine if these drugs are able to cross the blood-brain barrier and act as central agonists at the D-2 receptors.

METHOD

Adult male Wistar rats (Morini, S. Polo d'Enza, Reggio Emilia, Italy) weighing 300–340 g were used. The animals were housed in cages measuring 50 × 25 × 20 cm, eight animals per cage, with water and standard laboratory food freely available, at 22 ± 2°C with relative humidity of 60% and 12-h light-dark cycles (light on from 6 a.m. to 6 p.m.). All tests were performed between 9 and 12 a.m., in a sound-proof room. Animals were observed, four or five per group, in special glass observation cages where they were allowed 15 min to become accustomed to the new environment before treatment was begun. Immediately after IP injection of FDA 24, FDA 40 and FDA 27F, they were observed continuously for 1 h by experienced researchers unaware of the animals' treatment.

Doses of drugs refer to the weight of the salt. All the substances were dissolved in distilled water and injected at a constant volume of 2 ml/kg. Pretreatment IP with sulpiride and domperidone was carried out 20 min beforehand. Doses of the antagonists, chosen on the basis of previous experiments, did not modify per se the parameters in question. Controls were given the same

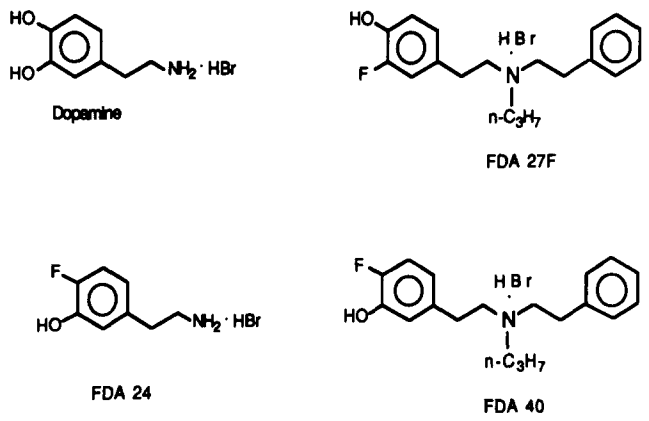

FIG. 1. Chemical structures of the 2-(fluorohydroxyphenyl)ethylamines tested in this study.

volume of vehicle. Each rat was used once only.

Animals exhibiting at least one episode of PE and SY during the test were considered as responding. Both the number of animals responding and the number of PE and SY episodes were recorded for each animal responding, as previously described (1). The results of PE and SY are presented as means per animal responding and as percentages of animals responding. Each experiment was performed at least twice so that no less than eight rats were employed for each treatment group. Statistical analyses were carried out by the two-tailed Mann-Whitney U-test or two-tailed Fisher exact test, with the level of significance set at $p<0.05$.

The following drugs were used: FDA 24: code number for 2-(4-fluoro-3-hydroxyphenyl)ethylamine hydrobromide; FDA 27F: code number for N-n-propyl-N-(2-phenylethyl)-2-(3-fluoro-4-hydroxyphenyl)ethylamine hydrobromide; FDA 40: code number for N-n-propyl-N-(2-phenylethyl)-2-(4-fluoro-3-hydroxyphenyl)ethylamine hydrobromide; L-sulpiride (Ravizza, Milan, Italy); and domperidone (Janssen, Beerse, Belgium).

RESULTS

As already reported, a low percentage of control animals exhibited sporadic episodes of PE (Fig. 2) and SY (Fig. 3) during the 1-h observation period.

FDA 40 and FDA 27F enhanced both signs (Figs. 2 and 3), increasing both the number of PE and SY per animal and the percentage of animals affected. While, in the case of FDA 27F, this stimulant activity appeared at only a few doses (5, 10 and 20 mg/kg), it was more marked for FDA 40 and covered a larger dosage range (from 1 to 40 mg/kg), the SY effect still being present in all rats (Fig. 3) at 40 mg/kg.

Of the three compounds tested, FDA 24 was definitely the least active. It failed to potentiate PE with respect to the controls (Fig. 2), while SY induction was of modest entity and, in our experimental conditions, detectable only at 20 mg/kg (Fig. 3).

Sulpiride pretreatment (10 and 20 mg/kg) significantly antagonized PE and SY induced by FDA 27F and FDA 40, while domperidone was completely ineffective (Table 1).

DISCUSSION

In our behavioural studies, FDA 24, FDA 27F and FDA 40 shared the ability to stimulate the SY-syndrome in rats, although the phenomenon varied in intensity according to the drug employed. Moreover, FDA 40 and FDA 27F were also able to enhance PE in rats. As already mentioned, these symptoms are typically induced by all DA-agonists at doses active on central D-2 receptors, and in our study were particularly evident after IP injection of FDA 40. This last compound produced a bell-shaped dose-response curve both for PE and SY, in that the effect was proportional to the dose up to the most active dose of 10 mg/kg, then higher doses were progressively less effective until the stimulation ceased.

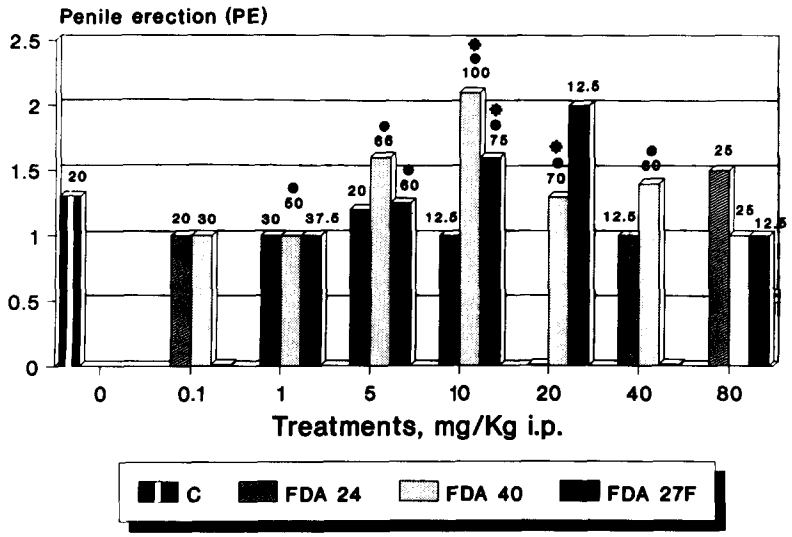

FIG. 2. Effect of FDA 40, FDA 27F and FDA 24 on penile erection (PE) in male rats. The drugs were injected immediately before the observation period (1 h). Each histogram represents the mean of PE per animal responding. The percentage of responding animals is reported above the histogram. ●Significantly different from controls (0) (Mann-Whitney U-test); *significantly different from controls (0) (Fisher exact test).

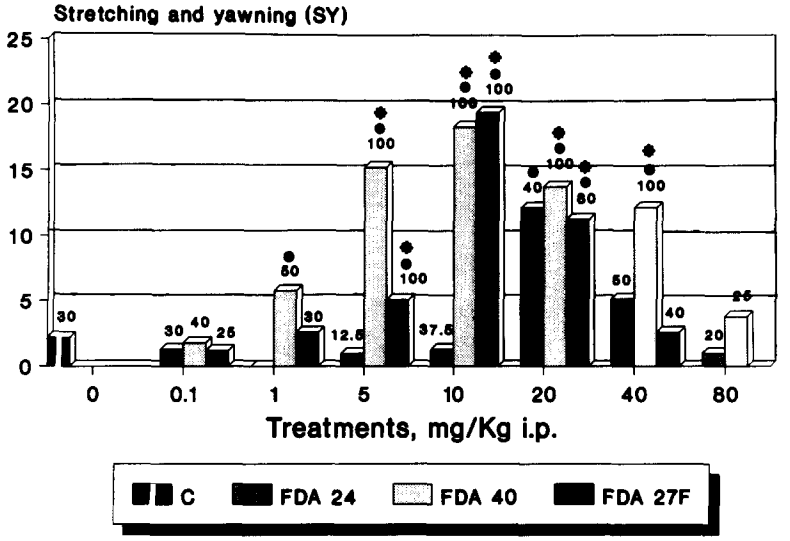

FIG. 3. Effect of FDA 40, FDA 27F and FDA 24 on stretching and yawning (SY) in male rats. The drugs were injected immediately before the observation period (1 h). Each histogram represents the mean of SY per animal responding. The percentage of responding animals is reported above the histogram. ●Significantly different from controls (0) (Mann-Whitney U-test); *significantly different from controls (0) (Fisher exact test).

The involvement of central D-2 DA receptors is supported by the antagonism exerted on PE and SY by pretreatment with sulpiride but not by domperidone, which does not cross the blood-brain barrier (15).

Along with the signs described here we also observed in the animals a concomitant state of sedation and no marked SB (data not reported). DA-agonist-induced hypomotility has been related to D-2 DA autoreceptor stimulation (6) where SB seems to be dependent on the cooperation of distinct D-1 and D-2 receptor systems (5). Irrespective of the location (pre- or postsynaptic), which at present is impossible to establish, our results for PE and SY and our preliminary behavioural observations on motility confirm

TABLE 1

INFLUENCE OF SULPIRIDE AND DOMPERIDONE ON PENILE ERECTION (PE) AND STRETCHING AND YAWNING (SY) INDUCED IN MALE RATS BY FDA 40 AND FDA 27F

Pretreatment (mg/kg)	Treatment (mg/kg)	Behavioural Effects PE	SY
—	—	1.2 ± 0.2 (20)	3.2 ± 0.3 (30)
—	FDA 40, 5	1.6 ± 0.4 (60)*	15.2 ± 2.3 (100)*
—	FDA 40, 10	2.6 ± 0.5 (90)*	16.2 ± 3.1 (100)*
sulp, 10	FDA 40, 10	1.0 ± 0.0 (50)	4.5 ± 1.2 (60)†
sulp, 20	FDA 40, 5	0.0†	2.3 ± 0.4 (40)†
sulp, 20	FDA 40, 10	1.0 ± 0.0 (30)†	3.0 ± 0.7 (50)†
domp, 3	FDA 40, 10	2.3 ± 0.4 (100)	20.7 ± 4.6 (100)
—	FDA 27F, 5	1.3 ± 0.2 (60)*	4.5 ± 0.6 (100)*
—	FDA 27F, 10	1.6 ± 0.4 (75)*	20.5 ± 6.2 (80)*
sulp, 10	FDA 27F, 5	1.0 ± 0.0 (20)†	1.6 ± 0.4 (20)†
sulp, 10	FDA 27F, 10	1.0 ± 0.0 (20)†	2.6 ± 1.0 (30)†
domp, 3	FDA 27F, 10	1.4 ± 0.2 (60)	21.4 ± 5.8 (70)

Either FDA 40 and FDA 27F were IP injected immediately before the observation period (1 h). Sulpiride (sulp) and domperidone (domp) were administered 20 min beforehand. Each value represents the mean ± SEM of PE and SY per animal responding. In parentheses the percentages of responding animals.

*Significantly different from control rats (Mann-Whitney U-test).

†Significantly different from rats treated with FDA 40 or FDA 27F alone at the same doses (Mann-Whitney U-test).

that FDA 27F and the more powerful FDA 40 are selectively active on D-2 DA receptors, in agreement with previous binding data. The reason why compounds specific for the D-2 receptors are being sought is that, since these receptors seem to be involved in various pathologies (10), any modification of their activity may be of clinical importance.

These results also provide evidence that the substitution of an hydroxyl group on the catechol moiety of DA with fluorine gives compounds that are able to cross the blood-brain barrier and act on the central D-2 receptors. The enhanced activity was obtained when the hydroxyl group and the fluorine atom on the phenyl ring are located at positions 3 and 4, respectively. Moreover, substitution of the amino group with n-propyl and 2-phenylethyl groups, which confer higher liposolubility on the molecules, intensifies the activity.

The perfect correspondence between the data obtained in binding studies and those found in behavioural experiments indicate that the PE and SY tests are sensitive and reliable tools for the study of DA-neurochemical mechanisms.

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