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SCH 23390 and its S-Enantiomer Stereoselectively Prevent EEG and Behavioral Activation Induced by Dopamine Agonists in the Rabbit

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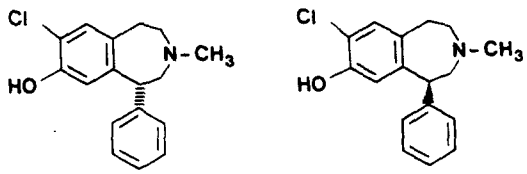
ONGINI, E., M. G. CAPORALI, M. MASSOTTI AND S. SAGRATELLA. *SCH 23390 and its S-enantiomer stereoselectively prevent EEG and behavioral activation induced by dopamine agonists in the rabbit.* PHARMACOL BIOCHEM BEHAV **26**(4) 715–718, 1987.—The selective D-1 dopamine antagonist SCH 23390 (R-enantiomer) and its unselective S-enantiomer (SCH 23388) were compared for their ability to prevent EEG and behavioral activation induced by the dopamine receptor agonists SKF 38393, apomorphine and LY 171555 in the rabbit. SCH 23390, at very low doses (0.003 mg/kg IV), inhibited EEG responses elicited by SKF 38393 and apomorphine, while the S-enantiomer displayed similar effects at doses at least 300-fold higher (1–3 mg/kg IV). Both isomers were approximately equipotent in preventing behavioral excitation caused by the D-2 agonist LY 171555. The dose of SCH 23390 interacting with LY 171555 was at least 100-fold higher than that effective for D-1 mediated responses. Conversely, the doses of S-enantiomer which prevented the stimulating effects induced by the different dopamine agonists were similar. The data demonstrate the stereoselectivity of the R-isomer SCH 23390 for blockade of D-1 receptors in vivo and provide evidence for the sensitivity of the EEG models in studying D-1 mediated responses.

D-1 antagonist SCH 23390 Benzazepine enantiomers Dopamine agonists EEG activity Behavioral arousal Rabbit

SCH 23390 and other closely related analogs of the benzazepine class are selective antagonists for D-1 dopamine receptors (for review, [2, 8, 9]). SCH 23390 was first identified as potent inhibitor of dopamine-sensitive adenylate cyclase, a marker for D-1 receptors, having weak affinity for ^{3}H-spiperone D-2 binding sites [6]. With the availability of the radioligand ^{3}H-SCH 23390, the selectivity of SCH 23390 itself and other benzazepines has been confirmed further [4,8]. One of the characteristics common to these compounds (Fig. 1) is a marked stereospecificity, with the R-enantiomer being more potent than the S-isomer [12]. SCH 23390 (Ki=0.3 nM) has a 640-fold greater affinity for D-1 binding sites than its S-enantiomer SCH 23388 (Ki=192 nM), whereas the two enantiomers are nearly equipotent in displacing ^{3}H-spiperone from D-2 binding sites (Ki=760 and 988 nM, respectively) [3,8].



R -enantiomer (SCH 23390) S -enantiomer (SCH 23388)

FIG. 1. Structures of the R-(active) and S-(inactive) enantiomers of the benzazepine D-1 antagonist 8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol.

The degree of selectivity for D-1 receptors exhibited by SCH 23390 in vitro (2500-fold) is however less evident in vivo. For example, the compound produces stimulation of prolactin secretion [1,8], which is identified as a D-2 receptor event, at doses at least 40-fold higher than those effective in responses involving D-1 receptors [8,14]. To interpret the paradoxical finding that SCH 23390 also induces D-2 effects, it has been hypothesized that a functional interaction exists between D-1 and D-2 receptors [3, 6, 11, 17, 20]. Furthermore, at certain dose levels it is likely that SCH 23390 interacts directly with D-2 receptors [1,18]. The narrow distinction in vivo between responses mediated by either D-1 or D-2 receptors makes it difficult to elucidate a functional role for D-1 receptors. In this regard, the availability of the pair of enantiomers having marked differences in affinity for D-1 receptors, but equally low potency for inhibition of D-2 re-

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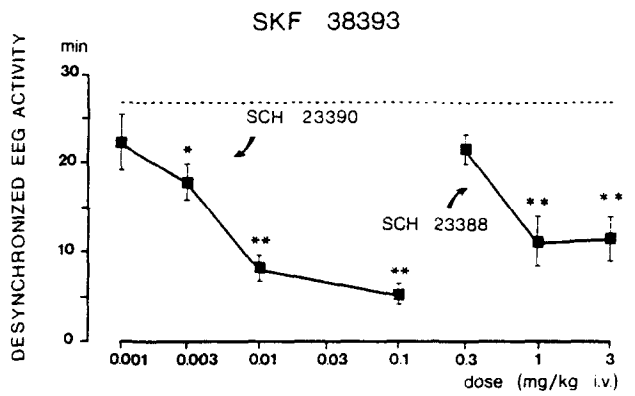

FIG. 2. Interaction of the R-enantiomer SCH 23390 and its S-isomer (SCH 23388) on EEG activation induced by SKF 38393 (10 mg/kg) in the rabbit. Each data point indicates min spent in the desynchronized EEG activity over 30 min, starting immediately after SKF 38393 injection. Both benzazepines were administered 15 min before SKF 38393. All drugs were given intravenously. $*p<0.05$,$**p<0.01$ compared to SKF 38393 alone (Dunnett's test).

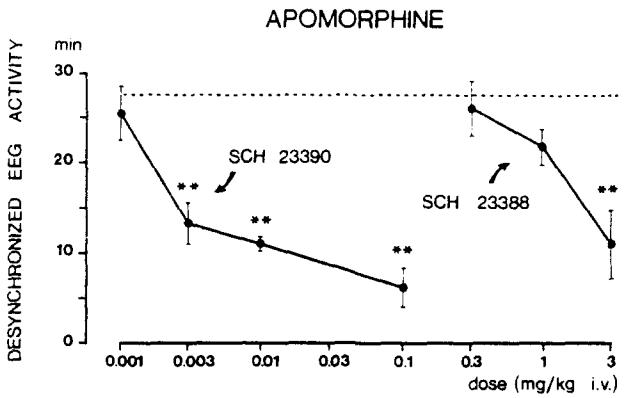

FIG. 3. Interaction of the R-enantiomer SCH 23390 and its S-isomer (SCH 23388) on EEG activation induced by apomorphine (1 mg/kg) in the rabbit. Other data as in Fig. 2. $**p<0.01$ compared to apomorphine alone (Dunnett's test).

ceptors, provides an interesting tool for better characterizing the D-1 antagonistic profile of SCH 23390.

Previous studies have shown that the R-enantiomer SCH 23390 potently blocks central effects induced by dopamine receptor agonists which stimulate D-1 receptor sites [14,15]. These studies have been extended to include the S-enantiomer SCH 23388. In the present experiments the two compounds were compared for their ability to interact with EEG and behavioral activation induced by SKF 38393, an agonist selective for D-1 receptors [19], by LY 171555, which stimulates D-2 receptors [21], and by apomorphine, an agonist for both receptor sites [4].

METHOD

Experimental Procedure

Experiments were performed on adult male rabbits weighing 2.2–2.7 g. Electrodes were implanted under local anesthesia (2% xylocaine), on the skull over the frontal, parietal and occipital cortices of both hemispheres [9]. A lateral ear vein was cannulated for drug administration. The animals were then partially restrained and accommodated in a sound-attenuated recording room. Experiments were started 1 hr thereafter.

EEG activity was displayed on an 8-channel Grass recorder (paper speed=15 mm/sec). EEG was continuously recorded for 1 hr prior to administration of either saline or drugs and for 1 hr thereafter. Evaluation of the recordings was made visually using standard criteria for recognition in the cortical leads of periods of desynchronization (low-amplitude fast-frequency waves) [10]. The time spent in the desynchronized activity was recorded for each 20-sec registration sheet. Data were grouped to provide information for segments of 10 min, 30 min and 1 hr. Duration of the EEG desynchronization for each treatment group during periods of 30 or 60 min was compared by means of two-way ANOVA followed by Dunnett's test for multiple comparisons.

Evaluation of behavioral responses to the administration of each agonist was limited by the experimental procedure used. Within these limitations, some behavioral changes were observed at 10-min intervals during the experiments. A state of arousal was recorded when animals displayed ears and head upright and eyes widely opened. Stereotyped behaviors, such as gnawing, sniffing or other repetitive perioral movements, were scored as absent, weak (periodic) or marked (continuous).

Drugs and Treatment

Groups of at least 5 rabbits received saline 15 min prior to the injection of SKF 38393 10 mg/kg, or apomorphine 1 mg/kg, or LY 171555 0.5 mg/kg to assess the standard response to each dopamine agonist. In separate experiments, either SCH 23388 (0.3–3 mg/kg) or SCH 23390 (0.003–0.3 mg/kg) was given 15 min prior to the agonist challenge. Each dose of the antagonist was evaluated in at least 4 animals. Other experiments were conducted to assess whether SCH 23388, over the dose range of 0.3–10 mg/kg, or SCH 23390 (0.1 mg/kg) induced EEG effects of its own.

Apomorphine hydrochloride (Sigma), SKF 38393 hydrochloride (Smith Kline and French Laboratories, USA) and LY 171555 (Eli Lilly and Company, USA) were dissolved in distilled water. SCH 23388 (Schering-Plough Corp., USA) was dissolved in HCl 0.1 N. SCH 23390 hemimaleate (Schering-Plough Corp., USA) was dissolved in 0.1% tartaric acid. All drugs were administered intravenously (IV) in a volume of 1 ml/kg. Doses are expressed in terms of the free base.

RESULTS

R-Enantiomer SCH 23390

Because both enantiomers exerted their prominent blocking action in the period of 30 min post-injection, all comparative analyses are referred to such a segment of EEG activity.

Given alone, SCH 23390, at the dose of 0.1 mg/kg, did not significantly affect EEG patterns. Over the 30 min post-injection the desynchronized activity was unaltered by the treatment with SCH 23390 (9.2±2.4 min compared to 10.4±1.9 min of controls, N.S.). In interaction experiments, SCH 23390 effectively blocked both EEG activation and be-

TABLE 1

THE TWO ENANTIOMERS SCH 23390 AND SCH 23388 DIFFERENTIALLY PREVENT EEG AND BEHAVIORAL ACTIVATION INDUCED BY DOPAMINE RECEPTOR AGONISTS

	Minimal Effective Dose (mg/kg IV)		
Agonist	R-Enantiomer SCH 23390	S-Enantiomer SCH 23388	Activity Ratio (S/R)
SKF 38393*	0.003	1	333
Apomorphine*	0.003	3	1000
LY 171555†	0.3	1	3

*Dose of enantiomer that significantly inhibited EEG activation for 30 min ($p<0.05$ Dunnett's test).

†Dose of enantiomer that attenuated the most typical behavior induced by LY 171555. Behavioral stereotypy was considered to be inhibited when only weak (periodic) or no effect was observed.

havioral arousal induced by the D-1 agonist SKF 38393, with a minimally effective dose of 0.003 mg/kg (Fig. 2). The same dose of SCH 23390 significantly prevented EEG activation (Fig. 3), and the typical behavioral reactions, such as compulsive gnawing, induced by apomorphine.

When EEG activation was induced by the D-2 agonist LY 171555, SCH 23390 did not exert any effects over the dose range of 0.003–0.3 mg/kg, $F(4.25)=2.21$, N.S. However, previous studies have shown that signs of behavioral stimulation induced by LY 171555, such as gnawing, back stretching and head movements are more sensitive to blockade of D-2 receptors than EEG activation [15]. Given at 0.3 mg/kg, SCH 23390 reduced the behavioral excitation typically elicited by LY 171555 from marked to episodic bursts of stereotyped behavior. Even at this high dose the antagonism by SCH 23390 was not complete in that some periodic signs of excitation remained apparent throughout the 1-hr test.

S-Enantiomer SCH 23388

SCH 23388 by itself did not substantially modify EEG patterns as compared to controls when administered over the dose range of 0.3–3 mg/kg, $F(3,18)=1.95$, N.S. Transient excitation was noted within 15 min after the administration of higher doses. At 3 mg/kg, EEG patterns were not substantially affected, whereas after the administration of 10 mg/kg there were high-amplitude slow-wave patterns or spike and wave complexes similar to those elicited by classical convulsants [5].

As shown in Fig. 2, SCH 23388 inhibited EEG activation induced by SKF 38393 over the dose range of 1–3 mg/kg. Similarly, SCH 23388 prevented apomorphine-induced EEG activation (Fig. 3) with a significant effect at 3 mg/kg. The marked behavioral effects typically produced by apomorphine [15] were not apparent in animals pretreated with SCH 23388 at 1–3 mg/kg. Conversely, EEG activation induced by LY 171555 was not significantly reduced by SCH 23388 0.3–3 mg/kg, $F(4,25)=2.75$, N.S. However, SCH 23388 at 1 mg/kg prevented LY 171555 from eliciting its typical behavioral stimulation, whereas the higher dose of 3 mg/kg was unable to attenuate LY 171555 effects substantially.

DISCUSSION

EEG activation induced by the D-1 agonist SKF 38393 [19] was prevented by minute doses of SCH 23390, the R-enantiomer (see also [14]). Conversely, behavioral actions initiated by LY 171555, a selective D-2 agonist [21], were influenced by SCH 23390 at doses at least 100-fold higher than those effective for blockade of D-1 receptors (Table 1) [15]. The data confirm further that, although to a lesser extent than in vitro [4,8], SCH 23390 retains its D-1 receptor selectivity when studied by using sensitive experimental paradigms in intact animals, e.g., EEG studies. In keeping with findings described by Gessa *et al.* [6], SCH 23390 also displayed a potent inhibition for EEG effects induced by apomorphine, a compound with equal affinity for both receptors [4]. This agrees with the concept that a functional interaction is operative between D-1 and D-2 receptor systems in several dopamine-mediated processes [2, 6, 13, 17, 20].

The S-enantiomer SCH 23388 was approximately equipotent in preventing stimulatory actions induced by the differently selective dopamine receptor agonists examined (Table 1). Over the dose range of 1–3 mg/kg, SCH 23388 interacted with EEG activation elicited by both SKF 38393 and apomorphine, a response mediated, either directly or indirectly, through D-1 receptors [15]. Similarly, SCH 23388 inhibited behavioral stimulation caused by the D-2 agonist LY 171555. It appears, therefore, that the results observed in vivo agree with in vitro findings showing lack of selectivity of the S-enantiomer for D-2 and D-1 binding sites [4,8]. Studies in the rat have shown that SCH 23388 is a weak compound in representative test paradigms such as blockade of conditioned avoidance responding and antagonism to apomorphine stereotypy [3,8]. Indeed, the compound by itself has little effect in vivo as well as weak affinity for both dopamine receptor subtypes in vitro. The data therefore indicate that the S-enantiomer deserves attention only as research tool for elucidating the mode of action of the active R-enantiomer SCH 23390.

Regarding the comparison between the two enantiomers, the data demonstrate that both compounds are nearly equipotent (3-fold separation) in interacting with D-2 mediated responses such as those elicited by LY 171555 (Table 1). A small separation in activity has also been reported for the two resolved enantiomers of the benzazepine D-1 antagonist SKF 83566 in tests of binding studies showing that the pair of enantiomers has approximately the same affinity in displacing ^{3}H-spiperone from D-2 binding sites [4,8]. Other studies have shown that the D-1 selective antagonist SCH 23390 can interact with D-2 agonists [11, 15, 17] and produce effects that occur through blockade of D-2 receptors [1,18]. However, most results have been observed with doses of SCH 23390 much higher than those selective for D-1 receptors. This is consistent with the view that SCH 23390, administered above certain dose levels, may influence D-2 receptor systems as a result of its weak affinity for such receptors [1,18]. Concerning this, the data obtained with both enantiomers appear to support the hypothesis that interactions with LY 171555 may be the net result of the equally weak affinity for D-2 binding sites. This does not rule out that the apparent D-2 responses displayed by SCH 23390 may also be attributed to the functional interactions existing between D-1 and D-2 receptor systems [3, 11, 13, 20]. The latter concept explains the evidence that SCH 23390 potently inhibits responses elicited by agonists for both receptor sites,

such as apomorphine [7,15], or compounds that stimulate dopaminergic systems non-selectively, such as amphetamine [7,8] or L-DOPA [16].

Differences between the two isomers were marked when stimulatory effects were induced by both SKF 38393 and apomorphine. In particular, the R-enantiomer SCH 23390 showed about 300-fold greater selectivity than its S-enantiomer in tests involving stimulation of D-1 receptors by SKF 38393. A similar high separation between the activity of the two enantiomers was also evident in interaction experiments with apomorphine, whose EEG effects are very sensitive to blockade of D-1 receptors by SCH 23390 [6,15]. The data are therefore consistent with the stereospecificity for D-1 binding sites displayed by the benzazepines SCH 23390 and analogues in vitro [8]. The selectivity manifested by SCH 23390, but not by its S-isomer, strongly supports the reliability of the EEG test paradigms in assessing D-1 mediated processes. Moreover, this study provides evidence for the usefulness of enantiomer pairs of benzazepine D-1 antagonists in experiments directed towards the understanding of the functional role of D-1 receptors.

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