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D-1 Agonist, SKF 38393, But Not a D-2 Agonist, Produces a Cholinergically Mediated Analeptic Effect in Rabbits

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HORITA, A. AND M. A. CARINO. *D-1 agonist, SKF 38393, but not a D-2 agonist, produces a cholinergically mediated analeptic effect in rabbits.* PHARMACOL BIOCHEM BEHAV **39**(2) 449–452, 1991.—SKF 38393 (2–15 mg/kg, IV), but not quinpirole, shortened the duration of loss of righting reflex produced in pentobarbital-narcotized rabbits. This effect was blocked by atropine (2–5 mg/kg, IV), but not by atropine methylbromide, suggesting that a central cholinergic mechanism was involved. The analeptic effect was also blocked by SCH 23390 (0.1 mg/kg, IV) or raclopride (5 mg/kg, IV). These results indicate that SKF 38393 activates central cholinergic neurons, which in turn initiate the analeptic effect. However, the fact that raclopride also blocked the SKF 38393 analeptic effect, but quinpirole did not exert any analeptic effect, suggests that a D-1/D-2 modulation of cholinergic systems may be involved in the SKF 38393-induced analeptic effect. These results also support our earlier findings and view that cocaine-induced analeptic activity is mediated by a dopaminergic-cholinergic mechanism.

D-1 D-2 SKF 38393 Quinpirole Analeptic Cholinergic

WE reported earlier that cocaine produced in pentobarbital-narcotized rabbits an analeptic effect (shortening of duration of loss of righting reflex) and EEG arousal (hippocampal theta) in rats, both of which were blocked by anticholinergic drugs (22). These results suggested the involvement of central cholinergic mechanisms, as has been reported for a variety of drugs and peptides possessing analeptic activity (7, 8, 10). In the case of cocaine, however, it was not clear whether the effect was produced directly on cholinergic neurons or whether it acted via a dopamine mechanism, since cocaine's psychoactive properties are mainly associated with DA uptake blockade (4, 17, 18).

We attempted to answer this question by examining the effects of D-1 and D-2 antagonists on the cocaine-induced analeptic effect. We found that SCH 23390, the D-1 specific antagonist, but not raclopride, the D-2 specific antagonist, blocked the cocaine effect, which suggested that cocaine produced its analeptic effect via an enhanced D-1 mechanism (23), presumably resulting from DA uptake blockade in central DA sites. From these data, we postulated that enhanced D-1 activity, either directly or indirectly, activated central cholinergic neurons (presumably hippocampal and/or cortical neurons), which in turn triggered the arousal (analeptic) response. In order to validate this hypothesis, we needed to demonstrate that a specific direct-acting D-1 agonist would, like cocaine, produce a cholinergically mediated analeptic effect in pentobarbital-narcotized rabbits. The present report describes such properties of the D-1 agonist, SKF 38393.

METHOD

Male New Zealand rabbits weighing 2.3–2.7 kg (R & R Rabbitry, Stanwood, WA) were used in these studies. All experiments were conducted in an ambient temperature of 22.0 ± 1.0°C.

The following drugs, the doses of which are expressed as the base, were dissolved in 0.9% saline solution: R(+)-SKF 38393 (RBI); (−)-quinpirole HCl (RBI); R(+)-SCH 23390 tartrate (Schering-Plough); raclopride tartrate (Astra); pentobarbital Na; atropine sulfate; atropine methyl bromide; oxotremorine HCl; and physostigmine salicylate. All injections in rabbits were made into a cannulated marginal ear vein.

The analeptic property of SKF 38393 was measured as a shortening of the duration (recovery of the righting reflex) of narcosis produced by pentobarbital. Integrity of the righting reflex was determined by placing the animal on its back and observing if it would resume and maintain the upright position. Injections and observations of animals were made by different individuals, i.e., the observer was not aware of the treatments given to each animal.

When the effects of drugs on physostigmine- or oxotremorine-induced excitation were examined, we employed an arbitrary rating scale in which the following criteria were used: behavioral rating 1 represented control (saline) animals in which the rabbits maintained a crouched prone position, were quiet,

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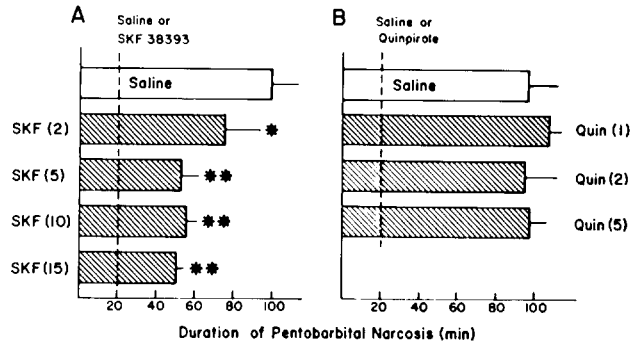

FIG. 1. Effect of varying doses of (A) SKF 38393 and (B) quinpirole on the duration of pentobarbital-induced narcosis in rabbits. Each bar represents the mean duration of narcosis (min ± SEM, N = 5–12) after the designated drug treatments. Saline or agonist was administered 20 min after pentobarbital. Figures in parentheses indicate dose (mg/kg IV) administered. One-way analysis of variance showed differences among the SFK 38393 (but not quinpirole) groups. $F(4,28) = 21.4$, $p<0.001$. $^*p<0.05$, $^{**}p<0.01$ when compared with saline controls, as determined by Newman-Keuls test.

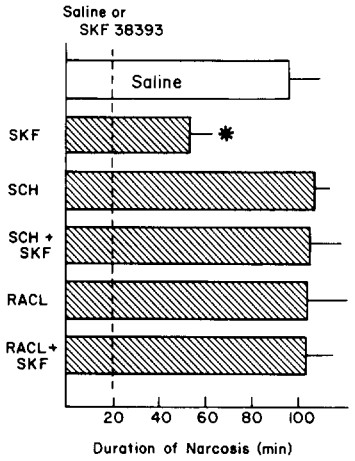

FIG. 2. Effect of SCH 23390 (0.1 mg/kg, IV) or raclopride (2 mg/kg, IV) on the analeptic effect of SKF 38393 (5 mg/kg, IV) in rabbits. Antagonist was given 10 min, and SKF 38393, 20 min, after pentobarbital. Each bar represents the mean duration of narcosis (min ± SEM, N = 5–12) after the designated drug treatments. One-way analysis of variance showed differences among groups. $F(5,37) = 7.44$, $p<0.005$. $^*p<0.01$ when compared with saline control as determined by Newman-Keuls test.

and exhibited no major activity or stereotypic movements. Behavioral rating 2 was given when the animals were alert and restless, maintained the crouched or sitting position but shifted from side to side, and showed occasional gnawing behavior. At a rating of 3, animals stood on all four legs, exhibited sporadic stomping of hind legs and occasionally circled within the box. They also showed a greater degree of stereotypic gnawing. Animals given a rating of 4 showed further intensification of rating 3 in which there was continuous movement and circling, hyperreactivity to tactile stimulation, and almost continuous gnawing behavior.

The observations of these behaviors were made with the animals placed in compartments measuring $10'' \times 15'' \times 12''$ high, large enough for them to circle, but not for running activity. All animals (except those receiving atropine) were pretreated with methylatropine to prevent development of the peripheral cholinergic side effects of physostigmine or oxotremorine.

The analeptic data were analyzed by one-way analysis of variance and the differences between treatment groups compared by the Newman-Keuls method. A difference of $p<0.05$ was considered statistically significant.

RESULTS

Analeptic Effect of SKF 38393

The analeptic effect of SKF 38393 in pentobarbital-narcotized rabbits is shown in Fig. 1A. One-way analysis of variance indicated a significant difference among the treatment groups, $F(4,28) = 21.4$, $p<0.001$. A dose of 2 mg/kg of SKF 38393 was active in reducing the duration of narcosis from 99 ± 14 min (controls) to 76 ± 19 min ($p<0.05$). With 5 mg/kg, narcosis time was further reduced to 54 ± 9 min ($p<0.01$), but higher doses of 10 and 15 mg/kg did not exert any greater analeptic activity. In contrast, quinpirole, the D-2 agonist, in doses of 1–5 mg/kg, was ineffective in exerting any analeptic activity (Fig. 1B). Larger doses of quinpirole were not used because those that were used were well within or greater than those needed to produce a variety of behavioral responses by other investigators (6,11).

Effect of D-1 and D-2 Antagonists on SKF 38393-Induced Analepsis

Figure 2 shows antagonism of the analeptic effect of SKF 38393 (5 mg/kg) by SCH 23390 (0.1 mg/kg) or raclopride (2 mg/kg). These doses of antagonists were selected because other investigators had used them for selective blockade of behaviors mediated by the respective receptor subtypes (13, 14, 16).

Effect of Atropine and Methylatropine on SKF 38393-Induced Analepsis

The cholinergic nature of the SKF 38393-induced analeptic effect is shown in Table 1. While atropine did not affect the duration of pentobarbital narcosis, it partially (2 mg/kg, $p<0.01$) or completely (5 mg/kg, $p<0.01$) blocked the analeptic effect of SKF 38393. In contrast, atropine methylbromide (methylatropine), a quaternary analog of atropine, was inactive in reversing the analeptic effect, indicating that a central cholinergic mechanism mediated the SKF 38393-induced analeptic effect.

Effect of Atropine, SCH 23390 and Raclopride on Oxotremorine- or Physostigmine-Induced Behaviors

The antagonism of SKF 38393 by atropine, SCH 23390 and raclopride suggested that both a dopaminergic and a cholinergic link were involved in the analeptic effect. However, since the blockade of the SKF 38393 effect by atropine or the dopamine antagonists were indistinguishable, it could be argued that the latter were acting like atropine as anticholinergic, rather than at dopamine sites. In order to clarify this question, we examined the effects of SCH 23390 and raclopride on the behavioral responses to the centrally acting cholinergic agents, oxotremorine (0.1 mg/kg) and physostigmine (0.2 mg/kg). At these doses, both compounds produced locomotor and stereotypic responses characterized by restlessness, stomping of hindlegs, hyperactivity and compulsive gnawing (behavioral rating 3). All animals (except the atropine group) were pretreated with atropine methylbromide (MAT) to protect the animals from the systemic effects of the cholinergic agonists. Figure 3 shows the effects of atropine, atropine methylbromide, SCH 23390 and raclopride on the behavioral effects produced by oxotremorine or physostig-

TABLE 1

ANALEPTIC EFFECT OF SKF 38393 (5 mg/kg IV) IN PENTOBARBITAL-NARCOTIZED RABBITS AND ITS REVERSAL BY ATROPINE (2 AND 5 mg/kg IV)

Drug Treatment				Mean Duration of Narcosis
1	2	3	N	(min ± SEM)
A. PB	Saline	Saline	14	98 ± 14
B. PB	Saline	SKF 38398	7	54 ± 9*
C. PB	Atrop (2)	Saline	5	105 ± 11
D. PB	Atrop (2)	SKF 38393	5	79 ± 12*
E. PB	Atrop (5)	Saline	8	109 ± 13
F. PB	Atrop (5)	SKF 38393	7	116 ± 13*
G. PB	AtrMeBr (5)	Saline	4	107 ± 10
H. PB	AtrMeBr (5)	SKF 38393	6	61 ± 6*

Drug 2 was administered 10 min, and drug 3, 20 min, after drug 1.

PB = Pentobarbital, 30 mg/kg, IV, Atrop = atropine (2 or 5 mg/kg, IV), AtrMeBr = Atropine methylbromide (5 mg/kg, IV). One-way analysis of variance showed differences among the treatment groups, $F(7,48) = 25.2$, $p<0.001$.

*$p<0.01$ when treatment B is compared to treatment A, treatment D is compared to treatment B, treatment F is compared to treatment B, and treatment H is compared to treatment A, as determined by Newman-Keuls test.

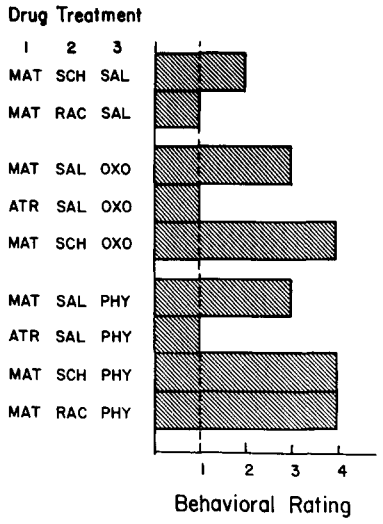

FIG. 3. Effect of atropine (ATR, 2 mg/kg), atropine methylbromide (MAT, 2 mg/kg), SCH 23390 (SCH, 0.2 mg/kg) or raclopride (RAC, 2 mg/kg) on the behavioral activating properties of oxotremorine (OXO, 0.1 mg/kg) or physostigmine (PHY, 0.2 mg/kg), all given IV. "Behavioral rating" is an arbitrary rating scale taking into consideration severity of locomotor and stereotypic responses produced by the cholinergic agonists under the conditions specified. Details of the rating scale are given in the Method section. Each bar represents mean responses observed in 3–6 animals. Dotted vertical line represents control saline (SAL + SAL + SAL) behavior.

mine. It is clear that, while atropine completely blocked oxotremorine or physostigmine effects, atropine methylbromide, SCH 23390 and raclopride were completely inactive, indicating that neither SCH 23390 nor raclopride possessed atropine-like anticholinergic activity. If anything, SCH 23390 and raclopride enhanced the behavioral effects of the cholinergic agents (behavioral rating 4).

DISCUSSION

We have demonstrated that SKF 38393, but not quinpirole, produced an analeptic effect in pentobarbital-narcotized rabbits. This effect was blocked by atropine as well as by the D-1 antagonist, SCH 23390. These results suggest that the analeptic effect of SKF 38393 is mediated via activation of central cholinergic mechanisms. It is unlikely that SCH 23390 is acting as an anticholinergic agent because it was incapable of reversing the behavioral effects of oxotremorine or physostigmine, which were completely blocked by atropine. Moreover, SCH 23390 has been reported to have little or no affinity for the muscarinic receptor (9).

These results are consistent with the EEG findings of Ongini et al. (12,13), who reported that SKF 38393 produced an arousal EEG in hippocampus and cortex of conscious rats and rabbits. However, these authors did not investigate the possibility of a cholinergic mechanism in these arousal responses. They found that quinpirole also produced EEG arousal, but their impression was that this effect might have been mediated indirectly because quinpirole produced considerable excitation and stereotypic behavior in conscious animals. Since our studies were conducted in animals under anesthesia, it was reasonable to assume that cholinergic activation by SKF 38393 mediated the analeptic response, whereas the lack of such activity by quinpirole could be explained on the basis that anesthesia prevented the behavioral excitation that was responsible for the arousal EEG seen in conscious animals.

These results also support our earlier findings that cocaine produced its analeptic and EEG arousal effects via a D-1-mediated activation of central cholinergic mechanisms, since they were also blocked by SCH 23390 as well as by scopolamine. The one difference between the cocaine-induced and SKF 38393-induced analeptic effects was that the latter was blocked by both SCH 23390 and raclopride, whereas the former was blocked only by SCH 23390. The antagonism of SKF 38393 arousal by raclopride was probably not produced by an anticholinergic or antiD-1 mechanism because raclopride lacks affinity for the muscarinic and D-1 receptors, respectively (5). While it is not clear as to why this difference between cocaine- and SKF 38393-induced analepsis exists, it may be related to cocaine exerting effects other than DA uptake blockade (such as norepinephrine and serotonin uptake blockade) that might contribute to the analeptic effect. This is not an unreasonable conjecture, since norepinephrine and serotonin are known to increase hippocampal ACh release (3,19). In contrast, based on its blockade by raclopride, the SKF 38393-induced analeptic effect may involve both D-1 and D-2 mechanisms, much like a number of other DA-mediated behavioral responses that exhibit a D-1/D-2 synergistic relationship (2, 20, 21).

We believe that these data are the first to demonstrate that a D-1 mechanism (as shown by SKF 38393) is involved in the activation of cholinergic systems that mediate the analeptic response. Earlier studies showed that apomorphine and other DA drugs inhibited septohippocampal cholinergic neurons via activation of a GABA mechanism (3). Based on our current understanding of DA receptor subtypes, it may be that this effect was mediated through D-2 receptors, since it was blocked by haloperidol (3). Other workers have reported that increased cortical ACh release was produced by administration of D-2 agonists, such as bromocriptine (15) and quinpirole (1), but not by SKF 38393. These data are inconsistent with our results, but the fact

that different species and anesthesia were used in our studies may explain this difference.

Thus there is growing evidence that DA mechanisms regulate hippocampal and cortical cholinergic activity. That such a relationship exists in the neostriatum is well known. Further research may clarify a similar DA-ACh relationship in modulating arousal and other behavioral functions associated with the hippocampal-cortical cholinergic systems.

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