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Modulation of (−)-sulpiride-induced increase in electrically-evoked release of dopamine from rat striatal slices

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Abstract—(−)-Sulpiride (10 nM–10 μM) in the superfusate, dose-dependently increased the electrically-evoked release of dopamine from rat striatal slices. (+)-Sulpiride had little effect on evoked release of dopamine up to 10 μM. Apomorphine inhibited electrically evoked release of dopamine, and this effect of apomorphine was antagonized by (−)-sulpiride. SCH23390 and forskolin had no effect on the (−)-sulpiride-induced increase in evoked release of dopamine. Treatment with the irreversible dopamine-receptor antagonist *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline caused a significant increase in evoked release of dopamine and prevented the (−)-sulpiride-induced increase in the evoked release of dopamine. These results indicate that the (−)-sulpiride-induced increase in evoked release of dopamine is due to antagonism of the activation of dopamine autoreceptors by endogenously released dopamine.

Micromolar concentrations of almost all neuroleptic drugs inhibit electrically-evoked release of dopamine from striatal slices (Dismukes & Mulder 1977; Miller & Friedhoff 1979; Yamada et al 1991) or striatal synaptosomes (Seeman & Lee 1975) of rats. However, (−)-sulpiride, even at high concentrations, increases [^{3}H]dopamine release from striatal slices in rats (Dwoskin & Zahniser 1986), and in rabbits (Starke et al 1978; Cubeddu et al 1983; Nowak et al 1983) and also increases the stimulating dopamine overflow observed with in-vivo voltammetry in the caudate-putamen (May & Wightman 1989). This effect is stereospecific and can be antagonized by apomorphine (Starke et al 1983), which indicates that dopamine autoreceptor blockade by (−)-sulpiride may account for the increase in the evoked release of dopamine. However, the precise mechanism underlying this phenomenon remains unclear. We studied the effects of apomorphine, SCH23390, forskolin, and *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an irreversible dopamine receptor blocker, on the sulpiride-induced increase in evoked release of dopamine from rat striatal slices.

Materials and methods

Male Wistar rats, 250–300 g, were housed in a light-, temperature-, and humidity-controlled environment for more than 2 weeks before the experiments. They were decapitated between 0900 and 1000 h and their brains were quickly removed. Some rats were treated intraperitoneally with 2 mg kg^{-1} of the peptide-coupling agent EEDQ (Sigma Co.) and the other control rats received 0·9% NaCl (saline).

Coronal sections, 0·3 mm thick, were sliced with a Micro Slicer (Dosaka E.M. Co.) from A 9760 to A 7630, according to the atlas of König & Klippel (1963), in ice-cold Krebs solution aerated with 95% O_2–5% CO_2. Striatal portions of the slices were punched out with a stainless steel tube (i.d. 4 mm). Each slice was placed in a chamber made from a teflon tube, with platinum electrodes at the top and bottom, then superfused with Krebs solution saturated with 95% O_2–5% CO_2 at 1 mL min^{-1} and 27°C. The composition of Krebs solution was as follows (mM); NaCl 118·0, KCl 4·9, $NaHCO_3$ 25, $NaHPO_4$ 1·25, $CaCl_2$ 1·25, $MgCl_2$ 1·18 and glucose 11·0, with nomifensine (3 μM).

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After 50 min of superfusion, the slice was stimulated with electric pulses of 1 Hz, 2 ms duration for 2 min (S_1). Then the slice was superfused with Krebs solution containing the drugs (apomorphine, SCH23390, forskolin, or (+)-sulpiride) with or without (−)-sulpiride. The second stimulation (S_2) was applied 30 min after the first, under the same conditions. Overflow superfusate was collected at 7 min intervals. Released dopamine in the superfusate was adsorbed with alumina, eluted with 300 μL 0·5 M acetic acid and measured by HPLC combined with electrochemical detection according to the method of Kissinger et al (1973), with a modification (Yamada et al 1991). The data were expressed as ratios of the amount of dopamine released by S_2 to that released by S_1, or as ng (mg protein)$^{-1}$ fraction of released dopamine. The data were subjected to analysis of variance followed by Scheffe's test.

Results

Concentrations of (−)-sulpiride greater than 1 mM slightly increased basal release of dopamine. Concentrations lower than 100 μM had no effect on basal dopamine release (data not shown). In contrast, the electrically-evoked release of dopamine was increased by superfusion with concentrations of (−)-sulpiride lower than 10 nM, and the effect was dose-dependent up to 1 μM (Fig. 1).

Forskolin, an adenylate cyclase activator that couples the D_1 receptor, caused a dose-dependent increase in basal and evoked release of dopamine. Superfusion with (−)-sulpiride increased the forskolin-induced increase in evoked release of dopamine, but the effect was additive (Table 1). The D_1-receptor blocker, SCH23390, had no effect on basal or evoked release of dopamine and had no effect on the sulpiride-induced increase in evoked release of dopamine (Fig. 1).

Apomorphine dose-dependently inhibited evoked release of dopamine. The ED50 value for the inhibitory effect of apomorphine was 0·3 μM. Superfusion with sulpiride, dose-dependently antagonized the inhibitory effect of 1 μM apomophine. (−)-Sulpiride (1 μM) completely reversed the reduction of evoked release of dopamine caused by 1 μM apomorphine (Table 2).

In rats given EEDQ (2 mg kg^{-1}, i.p.) 24 h before the experiment, there was significantly more evoked release of dopamine (3·04 ± 0·34 ng (mg protein)$^{-1}$/fraction, mean ± s.e.m, n = 12, $P < 0·01$) than in control rats (1·87 ± 0·23 ng (mg protein)$^{-1}$/fraction, mean ± s.em., n = 12). However, the S_2/S_1 ratio of the slices from rats given EEDQ was 1·34 ± 0·12 (mean ± s.e.m., n = 8), which was not significantly different from control group (1·42 ± 0·21, mean ± s.e.m., n = 8). In the slices from rats given saline, superfusion with 1 μM (−)-sulpiride caused a 171% increase in evoked release of dopamine. In the rats given EEDQ, (−)-sulpiride (1 μM) induced only a 135% increase in evoked release of dopamine. There was a statistically significant difference in the (−)-sulpiride-induced increase in evoked dopamine release between the EEDQ group and the control group. By contrast, superfusion with 1 μM apomorphine caused a 45% reduction of evoked dopamine release from the slices of rats given saline. The inhibitory effect of apomorphine on evoked release of dopamine was completely antagonized by EEDQ (Table 3).

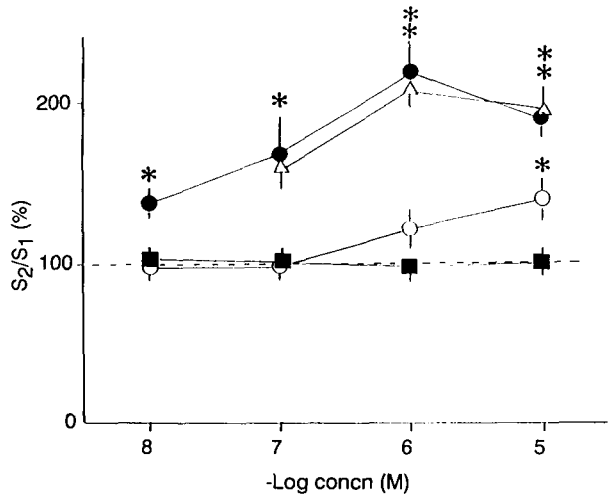

FIG. 1. Effects of (+)-, (−)-sulpiride and SCH23390 on electrically-evoked release of dopamine from striatal slices. The values are ratios of electrically-evoked release of dopamine with (S_2) and without (S_1) drugs. The results are expressed as mean ± s.e.m. (n = 5–8) at each point. *$P < 0.05$, **$P < 0.01$ compared with control S_2/S_1 ratio. ● (−)-sulpiride, ○ (+)-sulpiride, ■ SCH23390, △ (−)-sulpiride + SCH23390.

Table 1. Effects of forskolin on (−)-sulpiride-induced increase in evoked release of dopamine from rat striatal slices.

Drugs	Concn (μM)	S_2/S_1 ratio	n
No drug		1·15 ± 0·08	6
(−)-Sulpiride	1	2·37 ± 0·13** (206)	6
Forskolin	1	1·44 ± 0·16*	6
+(−)-Sulpiride	1	2·73 ± 0·11** (190)	6

Parentheses represent percent increases caused by (−)-sulpiride with or without forskolin. * $P < 0.05$, ** $P < 0.01$ compared with each control ratio.

Table 2. Effect of (−)-sulpiride on apomorphine-induced reduction in electrically-evoked release of dopamine.

Drugs	Concn (μM)	S_2/S_2 (% of control)	n
No drug		100	6
Apomorphine	1	40·2 ± 2·2**	6
Apomorphine (1 μM)			
+(−)-sulpiride	0·01	45·6 ± 2·8**	6
+(−)-sulpiride	0·1	63·4 ± 3·4*	6
+(−)-sulpiride	1	108·9 ± 10·4	6

*$P < 0.05$, **$P < 0.01$ compared with the control S_2/S_1 ratio.

Table 3. Effects of treatment with EEDQ on (−)-sulpiride-induced increase or apomorphine-induced reduction in evoked release of dopamine from striatal slices.

Treatment	Drugs	Concn (μM)	S_2/S_1 ratio	n
Saline	Control		1·42 ± 0·21	8
EEDQ	Control		1·34 ± 0·12	8
Saline	Apomorphine	1	0·78 ± 0·10**	6
EEDQ	Apomorphine	1	1·31 ± 0·13[a]	6
Saline	(−)-Sulpiride	1	2·44 ± 0·21**	6
EEDQ	(−) Sulpiride	1	1·81 ± 0·14[b]	6

**$P < 0.01$ compared with control, [a]$P < 0.01$ compared with 1 μM apomorphine, [b]$P < 0.05$ compared with 1 μM (−)-sulpiride control. Note that apomorphine-induced reduction and (−)-sulpiride-induced increase in evoked release of dopamine were both attenuated by EEDQ.

Discussion

The present data show that (−)-sulpiride stimulated the electrically-evoked release of dopamine from striatal slices of rats, without any effect on the basal release of dopamine. This agrees with previous findings that (−)-sulpiride increases electrically-evoked [^{3}H]dopamine release from slices of rabbit striatum (Nowak et al 1983), increases veratridine- and electrically stimulated release of endogenous dopamine from rat striatal slices (Herdon et al 1987) and increases Ca^{2+}-evoked release of [^{3}H]dopamine from striatal synaptosomes (Bowyer & Weiner 1987). The stimulatory effect of (−)-sulpiride on evoked release of dopamine was stereospecific and it antagonized the inhibitory effect of apomorphine on evoked release of dopamine, which agrees with previous reports (Arbilla & Langer 1981; Starke et al 1983; Parker & Cubeddu 1985; Herdon & Nahorski 1987; Herdon et al 1987). The data indicate that (−)-sulpiride had its effect at release-modulating dopamine autoreceptor sites.

Pretreatment with EEDQ, an irreversible dopamine-receptor blocker (Hamblin & Creese 1983; Meller et al 1985) can cause a marked decline in the number of D_2-receptor sites available for binding (Leff et al 1984), and a decline in the inhibitory effect of apomorphine on electrically-evoked [^{3}H]dopamine release from the rat striatum (Yokoo et al 1988). In the present study, EEDQ significantly increased evoked release of dopamine (S_1). The data indicate that electrically-evoked release of dopamine was inhibited by the activation of dopamine autoreceptors by endogenously released dopamine. Moreover, (−)-sulpiride-induced increases in evoked release of dopamine were reduced by EEDQ, and the inhibitory effects of apomorphine on the evoked release of dopamine were also attenuated by EEDQ. This is the first report describing the effect of treatment with EEDQ on (−)-sulpiride-induced increases in evoked release of dopamine. These findings indicate that EEDQ can prevent the effects of a dopamine agonist and also of a dopamine antagonist on evoked release of dopamine. Neither SCH23390 nor forskolin affected (−)-sulpiride-induced increases in evoked release of dopamine, which indicates that D_1 receptors do not contribute to the release-modulating effect of dopamine, in agreement with a previous report (Parker & Cubeddu 1985). These results are compatible with the previous findings that the release-modulating dopamine autoreceptor is a high-affinity D_2 (Lehmann et al 1983) or a D_3 receptor (Sokoloff et al 1990).

Taken together, these results indicate that the (−)-sulpiride-induced increases in evoked release of dopamine are due to antagonism of the activation of dopamine autoreceptors by endogenously released dopamine.

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Effect of chlorpromazine on mouse ambulatory activity sensitization caused by (+)-amphetamine

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Abstract—The development of sensitization to the ambulation-increasing effect of (+)-amphetamine (2·5 mg kg^{-1}) was found to be dose-dependently inhibited when 1 or 2 mg kg^{-1} chlorpromazine was administered concomitantly, and the sensitization to (+)-amphetamine was almost completely suppressed when co-administered with 4 mg kg^{-1} chlorpromazine. Following a challenge dose of 2·5 mg kg^{-1} (+)-amphetamine, mice pretreated with (+)-amphetamine alone or with (+)-amphetamine plus 1 or 2 mg kg^{-1} chlorpromazine showed similar marked enhancement of the sensitization. However, mice that had been given (+)-amphetamine plus 4 mg kg^{-1} chlorpromazine displayed only slight enhancement of the effect compared with the activity level in saline-pretreated mice.

Many investigators (Pickens & Crowder 1967; Rushton et al 1968; Tilson & Rech 1973; Segal & Mandell 1974; Short & Shuster 1976; Kokkinides & Zacharko 1980) who have studied the effects of repeated administration of amphetamines to animals have suggested enhancement of sensitivity to the ambulation-increasing and stereotypy-producing effects of the drug. However, our studies of sensitization to the ambulation-increasing effects of amphetamines (Tadokoro & Ohashi 1975; Hayashi et al 1980; Hirabayashi & Alam 1981) have indicated that this phenomenon is strongly affected by dose and interval of the administration, as well as by environment. Neuroleptics, such as chlorpromazine and haloperidol, are known to be effective in attenuating the stimulant effects of amphetamines (Sulser & Dingell 1967; Kuczenski & Leith 1981; Ihara 1983; Kashiwara et al 1984; Kuribara & Tadokoro 1985); the purpose of these experiments was to examine the mechanism by which chlorpromazine blocks sensitization to the ambulation-increasing effects of (+)-amphetamine.

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Materials and methods

Animals. Adult male dd strain mice, 24–32 g at the beginning of the experiment, were supplied by the Institute of Experimental