Effects of Benzamide Derivatives on Dopamine Release from Striatal Slices of Rats

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Received for publication October 23, 1993

Summary: The effects of the benzamide derivatives, (-)-sulpiride, sulfinapride and nemonapride, on spontaneous and electrically evoked dopamine (DA) release from striatal slices of rats were investigated. Nemonapride at concentrations of 100 nM or higher caused a dose-dependent increase in spontaneous DA release from the slices. (-)-Sulpiride and sulfinapride had little effect on spontaneous DA release. The electrically evoked DA release was enhanced by (-)-sulpiride at concentrations of 100 nM or higher in the superfusate and reduced by nemonapride at 100 nM or higher. Sulfinapride caused a small increase in the evoked release of DA. The different activities of these drugs on DA release may account for the different clinical results they produce.

Key words: dopamine release — rat striatum — (-)-sulpiride — sulfinapride — nemonapride

Introduction

Micro-molar concentrations of almost all neuroleptics inhibit electrically evoked dopamine (DA) release from striatal slices in vitro (Dismukes and Mulder, 1977; Yamada et al. 1991) and the potency for inhibition of evoked DA release correlated well with the daily dosages for clinical use (Seeman and Lee, 1975). However, (-)-sulpiride, even at high concentrations, enhanced the evoked DA release from striatal slices of rats (Dwoskin and Zahniser, 1986; Yamada et al. 1993) and rabbits (Starke et al. 1978; Cubeddu et al. 1983; Nowak et al. 1983) as well as the stimulated DA overflow observed with in vivo voltammetry in the caudate putamen (May and Wightman, 1989). This unique property of (-)-sulpiride may account for the antidepressant action of the drug, when a small dose is used in depressed patients.

In the present study, the effects of other benzamide derivatives, nemonapride and sulfinapride, on spontaneous or electrically evoked release of DA were studied and compared to the effects of (-)-sulpiride to establish whether the stimulating effect of (-)-sulpiride on evoked DA release is derivative specific.

Materials and Methods

Male Wistar rats (250-300 g) were used for all experiments. Animals were housed in a light-, temperature- and humidity-
controlled environment. The brain was quickly removed after anesthesia. Coro-
nal sections were made with a Micro Slicer (Dosaka EM Co.) in ice cold Krebs
solution aerated with 95% O2 and 5% CO2. The striatal portion of each slice
was punched out with a stainless steel tube (i.d., 4 mm). One slice of striatum
was placed in a chamber made from a teflon tube with platinum electrodes at
the top and bottom to stimulate the slice with electric pulses. The slice was super-
fused with Krebs solution saturated with 95% O2 and 5% CO2 at a flow rate of
0.7 ml/min at 37°C. The composition of the Krebs solution was as follows (in
mM); NaCl, 118.0; KCl, 4.9; NaHCO3; 25.0; NaHPO4, 1.25; CaCl2, 1.25; MgCl2,
1.18; glucose, 11.0; and nomifensin (3 µM). Following superfusion for 50 min,
the slices were stimulated with electric pulses at 1 Hz frequency and 2 ms dura-
tion for 1 min (S1). Krebs solution containing various concentrations of (−)
sulpiride, sultopride or nemonapride was then applied. A second stimulation (S2)
was performed, 30 min after the first stimulation, using the same stimulation
parameters. The overflowing superfusate was collected before and after each stim-
ulation period at 7 min intervals. The release evoked during the S1 and S2 pe-
riods of stimulation were estimated from the amounts of total minus spontaneous
release during each stimulation. The spontaneous release during S1 and S2 were
estimated from the amounts released for the 7 min immediately before the stim-
ulation and are represented by sp1 and sp2. The released DA in the superfusate
was adsorbed by 50 mg alumina, eluted with 300 µl of 0.5 N acetic acid and quan-
tified by high performance liquid chromatography with electrochemical detec-
tion according to the method of Kissinger et al. (1972) with slight modifications.
The data are expressed as the ratio of the amount of DA released by sp1 vs sp2
or S1 vs S2 or ng/mg protein/fraction. The statistical comparisons were per-
formed using ANOVA followed by a Scheffe's test.

Results

Effects of benzamide derivatives on spontaneous release of DA from striatal slices of rats

The spontaneous release of DA was 0.31±0.02 ng/mg protein per 7 min fraction,
which changed by less than 5% over 70 min.

As shown in Fig. 1, (−)-sulpiride even at concentrations higher than 100 µM had
no effect on the spontaneous release of DA. Nemonapride (100 nM or higher) in-
duced a dose-dependent increase in spontaneous release of DA from the striatal
slices. Sultopride at concentrations of 10

![Fig. 1. Effects of benzamide derivatives on spontaneous dopamine release from striatal slices of rats. The data represent mean %±S.E.M. of the sp2/sp1 ratio at various concentrations of (−)-sulpiride (■), sultopride (●) or nemonapride (○) (n=6-10 for each point; *P<0.05, **P<0.01, when compared with the control sp2/sp1 ratio. An ANOVA followed by a Scheffe's test was used).](image-url)
EFFECTS OF BENZAMIDE DERIVATIVES ON DOPAMINE RELEASE

Fig. 2. Effects of benzamide derivatives on electrically evoked dopamine release from striatal slices of rats. The data represent mean ±S.E.M. of the control S2/S1 ratio at various concentrations of (−)-sulpiride (○), sultopride (●) or nemonapride (□). (n=6-10 for each point; *P<0.05, **P<0.01, when compared with the control S2/S1 ratio. An ANOVA followed by a Scheffe’s test was used.)

µM or higher caused a small but significant increase in the spontaneous release of DA.

Effects of benzamide derivatives on electrically evoked release of DA from striatal slices

The electrically evoked DA release was 2.11±0.16 ng/mg protein per 7 min fraction which was 7 times greater than the spontaneous release. The control S2/S1 ratio was 1.22±0.12 (mean ±SEM, n=21). (−)-Sulpiride caused a concentration-dependent increase in the evoked DA release at concentrations higher than 100 nM. The ED50 was 3 µM. In contrast, nemonapride caused a concentration-dependent decrease in the evoked DA release from the slices. Sultopride caused a small but significant increase in the evoked DA release (Fig. 2).

Discussion

Nemonapride caused a dose-dependent increase in spontaneous DA release from the striatal slices. Haloperidol has been reported to have a stimulatory effect on spontaneous DA release from striatal slices of the rat. The EC50 for haloperidol was 26 µM (Yamada et al. 1990). The potency of nemonapride in stimulating spontaneous release of DA was almost the same as that of haloperidol. It has previously been reported that the haloperidol-induced DA release was attenuated by atropine and enhanced by physostigmine. Thus, the haloperidol-induced release of DA is, at least partially, mediated by the activation of muscarinic ACh receptors located in the striatum (Yamada et al. 1990). The present results indicate that the nemonapride-induced increase in spontaneous release of DA is mediated by the same mechanism as the haloperidol-induced increase in the release of DA. (−)-Sulpiride up to 100 µM had no stimulatory effect on the spontaneous release of DA from the slices. (−)-Sulpiride caused a dose-dependent increase in the evoked release of DA from the slices at concentrations of 100 nM or higher which is in agreement with previous reports (Cubeddu et al. 1983; Nowak et al. 1983; Dwoskin and Zahniser, 1986). The (−)-sulpiride-induced increase in the evoked release of DA was attenuated by superfusion with apomorphine or pretreatment with EEDQ, an irreversible D2 receptor blocker (Yamada et al. 1993), which indicates that the activation of DA autoreceptors by endogenous DA is blocked by (−)-sulpiride, resulting in an enhancement of the evoked release of DA. Although nemonapride (Shibanoki et al. 1989; Terai et al. 1989; Wanibuchi and Usuda, 1990) and sultopride (Jenner et al. 1978) are also selective antagonists of D2 receptors, a stimulatory effect of nemonapride on the evoked release of DA.
was never observed at any concentration (Fig. 2). Sultopride caused a small but significant increase in the evoked release of DA. On the contrary, nemonapride at 100 nM or higher markedly inhibited the electrically evoked release of DA (Fig. 2) and this action was almost as strong as that of haloperidol (Yamada et al. 1991). Thus, the effects of nemonapride on the evoked and spontaneous release of DA were similar to those of haloperidol. The mechanism underlying the inhibitory effect of nemonapride on the evoked release of DA remains unknown. Mechanisms other than D2 receptor block can be suggested. First, most neuroleptics, but not (−)-sulpiride are highly lipophilic and may therefore produce non-specific perturbations of membrane lipids at high concentrations, resulting in an inhibition of the evoked release of DA. Second, (−)-sulpiride is highly selective for the D2 receptor, but nemonapride inhibits 3H-ketanserin binding to 5-HT2 sites as well as 3H-spiperon binding to D2 sites and both are also inhibited by haloperidol (Chivers et al. 1988). Thus the 5-HT2 receptor blocking action of these drugs may account for the inhibitory effect on the evoked release of DA.

The present results indicate that the (−)-sulpiride-induced increase in the evoked release of DA is not derivative specific. These benzamide derivatives have clinical features that are quite different from each other. The different effects of these drugs on evoked and spontaneous release of DA from striatal slices may be the basis for the different pharmacological properties of the drugs. The stimulatory effect of (−)-sulpiride on evoked DA release may be especially important for the antidepressant effect of the drug.

References


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