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Inhibition of Evoked Dopamine Release by Monopropionylcadaverine *in Vitro*[1]

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SALZMAN, S. K. AND M. STEPITA-KLAUCO. *Inhibition of evoked dopamine release by monopropionylcadaverine* in vitro. PHARMAC. BIOCHEM. BEHAV. **15**(1) 119–123, 1981.—A naturally occurring diamine, cadaverine, and one of its acyl derivatives, monopropionylcadaverine, were tested for their effects on the *in vitro* release of endogenous dopamine from slices of the rat neostriatum. Dopamine release was allowed to occur spontaneously and was evoked by elevating the potassium concentration in the incubation medium or by electric field stimulation. Monopropionylcadaverine had no effect on spontaneous release of dopamine and little effect on potassium-evoked release of dopamine, but at concentrations as low as 10^{-8} M in the medium it significantly depressed the electrically induced dopamine release.

Monopropionylcadaverine Cadaverine Dopamine release inhibition

CADAVERINE (1,5-diaminopentane) is formed in mammalian tissue by decarboxylation of lysine, probably through enzymatic conversion by ornithine decarboxylase [13, 22, 24]. The synthesis of cadaverine appears to be maximal under conditions of rapid tissue proliferation such as during development [6], anabolic steroid administration [13], and pregnancy [1].

Monopropionyl- and monoacetylcadaverine are believed to be the metabolic products of the acylation of cadaverine [32]. Cadaverine is acylated, predominantly to the propionyl form, after intraventricular injection in the rat brain [28]. The acyl derivatives of cadaverine were elevated in the urine [23] and blood [10] of schizophrenics as compared with normal and institutionalized control subjects. The speculation is that these elevations may result from lowered monoamine oxidase (MAO) activity because administration of MAO inhibitors significantly elevates the concentrations of the monoacylcadaverines in the blood and brain of mice [11] and rats [8]. MAO activity in white cells and platelets was lowered in a subgroup of chronic schizophrenics (for review see [33]).

We were interested in determining whether the elevated levels of monoacylcadaverines interfered with functions of the central nervous system. We had found that iontophoretic application of monopropionylcadaverine onto molluscan central neurons resulted in a long-lasting depolarization of the neuronal membrane. Cadaverine did not have this effect [20]. We also found that chronic elevation of the levels of monoacetylcadaverine in the blood of rats caused specific alterations in the average duration of nonrapid eye movement (NREM) sleep [4,27]. The same effects, but of greater magnitude, were seen after acute intraventricular injection of much lower doses of monoacetylcadaverine. Similar effects on NREM sleep duration have been seen after intravenous infusion of low doses of the dopamine agonist apomorphine [18].

The purpose of this study was to determine whether the pharmacological activity of monoacylcadaverines could be mediated through the dopaminergic system, perhaps providing a relationship between the apparent elevations of monoacylcadaverines in the blood of schizophrenics and the dopamine hypothesis of the etiopathology of schizophrenia [30]. Fluphenazine, a neuroleptic drug and dopamine receptor antagonist, was also tested for its ability to alter release of dopamine.

METHOD

The pharmacological activities of cadaverine, its monopropionyl derivative, and fluphenazine were determined in tests using slices of the rat neostriatum. Brain slices in oxygenated physiological buffers are believed to retain the metabolic and electrical characteristics of neuronal tissue for several minutes [14, 17, 34]. The release of endogenous dopamine from the slices was monitored by assaying aliquots of the buffer by high-pressure liquid chromatography with electrochemical detection (Bioanalytical Systems). The technique has been described previously in detail [25,26].

Male black-hooded rats of the Long-Evans strain (300–350 g) were killed by cervical dislocation, and their brains were rapidly removed. The striata were dissected out and sliced free-hand. During the dissection, tissues were con-

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TABLE 1

EFFECTS OF MONOPROPIONYLCADAVERINE, CADAVERINE, AND FLUPHENAZINE ON POTASSIUM-EVOKED RELEASE OF DOPAMINE FROM SLICES OF THE RAT NEOSTRIATUM

	Normal Saline	High-Potassium Saline					
			Monopropionylcadaverine			Cadaverine	Fluphenazine
Incubation time	No Drug N=12	No Drug N=12	10^{-4} M N=12	10^{-6} M N=12	10^{-8} M N=12	10^{-6} M N=6	10^{-5} M N=12
15 min	2.44 ± 0.72*	4.67 ± 0.86	1.54 ± 1.00*	3.19 ± 0.80†	4.07 ± 1.04	4.17 ± 1.11	5.52 ± 1.62‡
30 min	0.80 ± 0.18§	2.13 ± 1.14	0.48 ± 0.29‡	1.43 ± 0.74	2.13 ± 1.10	2.04 ± 1.02	3.87 ± 1.20‡
45 min	0.89 ± 0.20§	1.52 ± 0.45	0.58 ± 0.39	1.01 ± 0.54¶	1.27 ± 0.48	1.47 ± 0.59	2.81 ± 0.22*
60 min	0.80 ± 0.34	1.55 ± 1.35	0.47 ± 0.25§	0.55 ± 0.13¶	1.14 ± 0.55	1.42 ± 0.77	2.83 ± 0.54‡

*Different from no-drug, high-potassium saline group at $p<0.001$ by Scheffé's range test.
†Different from no-drug, high-potassium saline group at $p<0.01$ by Student's t-test (two-tailed); no significance by Scheffé's range test.
‡Different from no-drug, high-potassium saline group at $p<0.01$ by Scheffé's range test.
§Different from no-drug, high-potassium saline group at $p<0.05$ by Scheffé's range test.
¶Different from no-drug, high-potassium saline group at $p<0.05$ by Student's t-test (two-tailed); no significance by Scheffé's range test.

tinuously irrigated with cold Yamamoto buffer [34]. The slices (average weight 61 ± 1 mg (SEM), N=186) were then placed in 1-ml glass tubes containing 500 μl of the buffer (37°C, aerated for 1 hr with 95% O_2/5% CO_2); the tubes were capped and placed in a shaking water bath at 37°C. The incubation medium was either normal physiological buffer or buffer containing an elevated potassium concentration (55 mM) in place of sodium. Tests were conducted using both the normal and high-potassium buffers, either alone or containing each of the following: 10^{-4}, 10^{-6}, and 10^{-8} M monopropionylcadaverine·HCl (KOR Isotopes); 10^{-6} M cadaverine·diHCl (Sigma); and 10^{-5} M fluphenazine diHCl (E. R. Squibb).

Dopamine release was allowed to occur spontaneously or was evoked by the high concentration of potassium or electrical stimulation. In the experiments using potassium-evoked release, 200-μl aliquots of the buffer were removed and assayed at 15-min intervals for up to 1 hr; 200-μl aliquots of the same buffer were always added back. In the experiments using electrically evoked release, the slices were placed in nylon-mesh bags and incubated for 15 min. They were then transferred to a stimulation chamber in which an electric field was applied for 1 min between concentric gold electrodes powered from a Grass S-8 stimulator (50-V, 10-Hz, 20-msec pulses). The tissue was then returned to its original container and sampled 15 min later. Slices were exposed to the various compounds during both pre- and post-stimulation periods.

The dopamine concentration was measured in the untreated buffer. Norepinephrine (Sigma Chem. Co.), which was not detected as an endogenous component in these experiments, was used as internal standard, and 20-μl aliquots of the sample were valve-loaded onto a pellicular strong cation exchance column (Vydac). The degassed mobile phase (0.1 M citrate-acetate buffer, pH 5.2) was pumped through the system at a flow rate of 0.8 ml/min. Norepinephrine and dopamine eluted from the column approximately 5 min and 10.7 min, respectively, after injection. The eluted catecholamines underwent oxidation to their corresponding quinones at the glassy carbon working electrode, which was located in a thin-layer flow cell at the outflow of the column. A potentiostat controlled the working potential (preset to 720 mV) and sensed the oxidation current (proportional to the catecholamine concentration), which was registered on a pen recorder. Dopamine was quantitated from an external standard curve generated for each experiment. Varying amounts of dopamine (Sigma Chemical Co.) were placed in 0.1 N HCl and injected in 20-μl aliquots onto the chromatograph. Dopamine release was estimated as the difference in dopamine concentration at the end of successive 15-min intervals, taking into account the amount removed for analysis. Results were expressed as picomoles of dopamine/mg striatum/15 min. Differences between experimental groups were determined by analysis of variance and range-testing by the Scheffé procedure [29].

RESULTS

The release of dopamine from brain slices can be potentiated by electric stimulation or by increasing the extracellular concentration of potassium ions. The term "evoked release" refers to the difference between the amount released with stimulation and that without it.

As Table 1 shows, the spontaneous and potassium-evoked release of dopamine during the first 15 min of incubation are very close to previously reported values of endogenous dopamine release in striatal slices [15, 19, 25]. The released amount of dopamine falls to a lower but stable value over the next 45 min of incubation. The values at 30 to 60 min are probably more accurate than the 15-min value in reflecting dopamine release because diffusion of dopamine down a concentration gradient is reduced. In the initial incubation period, dopamine is released into a medium containing no dopamine; most likely a portion of this apparent release is due to the passive diffusion of dopamine.

As shown in Fig. 1, none of the three compounds had any significant effect on spontaneous dopamine release (i.e., in normal buffer).

Dopamine release was significantly increased ($p<0.001$) by electrical stimulation (Fig. 2, second column vs first). All three concentrations of monopropionylcadaverine significantly inhibited this increase. The amount of dopamine released by the stimulation was cancelled by a concentration of 10^{-4} M and attenuated by 10^{-6} M and 10^{-8} M monopropionylcadaverine. Fluphenazine significantly increased the electrically evoked release of dopamine. Cadaverine (at 10^{-6} M) was inactive (not shown).

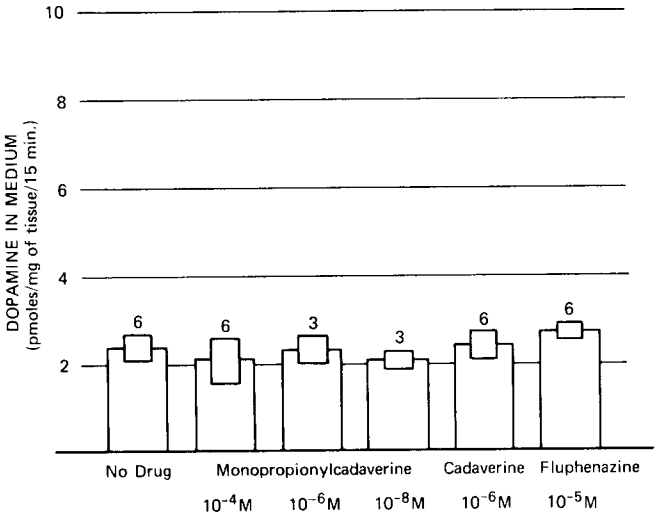

FIG. 1. Effect of monopropionylcadaverine, cadaverine, and fluphenazine on spontaneous dopamine release from striatal slices of the rat brain at 15 min of incubation. Values are means±SEM. The number of slices is indicated above each column, and the conditions of treatment are given on abscissa. Differences between means were tested by the analysis of variance.

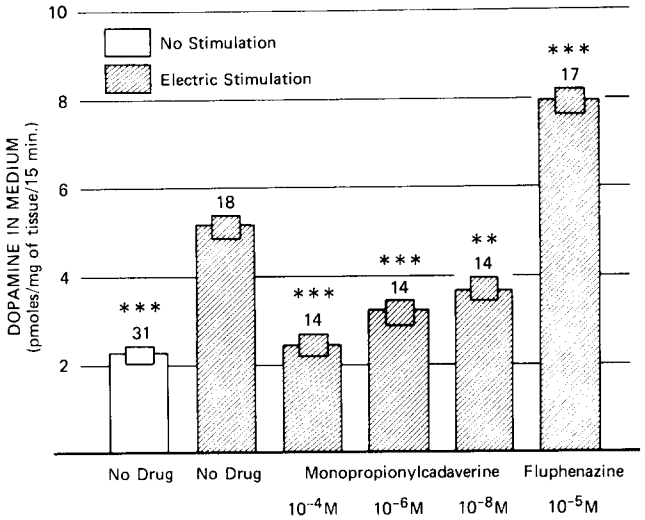

FIG. 2. Effect of monopropionylcadaverine and fluphenazine on dopamine release from striatal slices of the rat brain evoked by electric stimulation (shaded columns, 50-V, 20-msec pulses at 10 Hz for 1 min; unshaded column, controls) after incubation for 15 min. Values are means±SEM. The number of slices is indicated above each column, and the conditions of treatment are given on the abscissa. Differences between means were tested by the analysis of variance. Their significance (as obtained by Scheffé's range procedure) relative to the mean shown in the second column is indicated by the number of asterisks above each column (**$p<0.01$; ***$p<0.001$).

The values for spontaneous and electrically induced dopamine release are similar to previously reported values [25].

In the potassium release experiment (Table 1), the amount of dopamine released by high potassium in the control medium was significantly different from the amount of dopamine released in 10^{-4} M monopropionylcadaverine or in 10^{-5} M fluphenazine by the high-potassium concentration. Potassium-evoked release in the presence of 10^{-8} M monopropionylcadaverine was not altered. Monopropionylcadaverine at 10^{-6} M slightly reduced the amount of evoked dopamine release in the high potassium medium; the reduction was significant by the Student's t-test, but was not significant by the Scheffé test.

Figure 3 shows the percentage of inhibition of stimulated dopamine release as a function of the dose of monopropionylcadaverine. Curves A through D are drawn through the dose-reponse values for the potassium-release experiments. Curve E represents values in the electrical-stimulation experiment. All the curves except D (high potassium at 60 min) show the steepest response between 10^{-6} M and 10^{-4} M concentrations of monopropionylcadaverine.

DISCUSSION

The release of catecholamines from neuronal endings is believed to be modulated by presynaptic receptors or autoreceptors [7]. Agonists cause a negative feedback diminution of transmitter released upon subsequent impluse activity, whereas antagonists increase the amount of stimulus-induced transmitter release. Fluphenazine, a dopamine receptor antagonist, increased the potassium-evoked and electrically stimulated release of dopamine from striatal slices. This observation is consistent with its ability to block the presynaptic effects of dopamine [21] and agrees with previous studies *in vivo* [9] and *in vitro* [31].

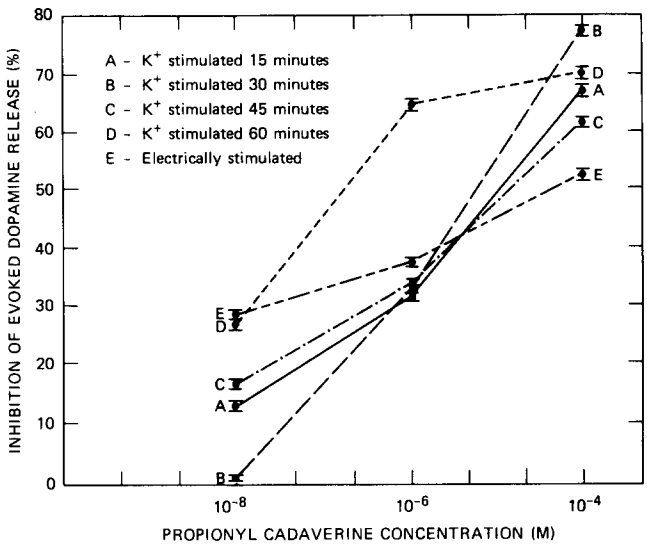

FIG. 3. Dose-response curves showing effect of three concentrations of monopropionylcadaverine on potassium-evoked or electrically stimulated release of dopamine. The values are the percentages of inhibition (means±SEM) of the evoked release.

Monopropionylcadaverine, however, had the opposite effect and hence may have been acting functionally as a dopamine agonist in this system [2]. That is, monopropionylcadaverine attenuated the evoked release of dopamine as a presynaptic agonist would be expected to do. Of course, this

effect may have been ultimately due to either an accelerated reuptake or a reduced synthesis of dopamine. The fact that the effects of monopropionylcadaverine were dependent on the procedure used to evoke dopamine release (i.e., elevating potassium vs electrical stimulation) mitigates against those possibilities. The lowest concentration of monopropionylcadaverine (10^{-8} M) was effective in inhibiting the electrically induced, but not the potassium-evoked, release of dopamine. Both procedures should cause neuronal depolarization. The augmentation of release by both procedures was similar (Table 1, Fig. 2). If monopropionylcadaverine was influencing the reuptake of dopamine already released into the medium, one would expect to see effects independent of the procedure used to evoke the release. Although both techniques have been used interchangeably in the past, it is not certain whether the release that each evokes is from the same or different transmitter pools. Recent evidence indicates that potassium-evoked release of transmitter is from a nonvesicular pool [3,15]. If this is so, it may indicate that monopropionylcadaverine—at least at concentrations of 10^{-4} M and 10^{-6} M—inhibits both vesicular and nonvesicular release of dopamine.

Another possibility may lie in the time course of release and subsequent changes in dopamine synthesis induced by each stimulation procedure. That is, the increased dopamine release induced by electric stimulation may be dependent on a subsequent, prolonged enhancement of dopamine synthesis. The apparent attenuation of this evoked release by monopropionylcadaverine then could be due to an inhibition of dopamine synthesis. The prolonged periods during which the slices were exposed to drug and the rather lengthy sampling periods (i.e., 15 min) make this a distinct possibility. Further studies on the time course of both electrically evoked and potassium-induced dopamine release and the alterations in dopamine synthesis induced by these procedures are necessary.

Apomorphine, which in low doses is believed to predominantly bind to presynaptic dopamine receptors [16], has the same effect as monopropionylcadaverine on the duration of NREM sleep in rats [18,27] and the stimulus-evoked release of dopamine from striatal slices [5]. On the basis of these findings, it is not unreasonable to assume that monopropionylcadaverine is an endogenous compound with at least presynaptic dopamine agonist-like properties, which are reflected ultimately in alterations in synaptically available dopamine. Coupled with the fact that acylcadaverines in the blood have access to the brain ([27] and Salzman and Stepita-Klauco, in preparation), the elevated levels of acylcadaverines seen in the blood of schizophrenics might lead to alterations in central dopamine neurotransmission. A recent report demonstrates that blood levels of monoacylcadaverines in schizophrenics are relatively higher during aggravation of clinical symptoms and lower during improvement of the illness [12].

The average concentration of monopropionylcadaverine in the whole mouse brain is about 3×10^{-9} M [11]. Some regions of the brain almost certainly contain monopropionylcadaverine in concentrations higher than the average for the whole brain. If the concentration of monopropionylcadaverine reaches 10^{-8} M in some brain regions, it can significantly lower the amount of dopamine released there as a result of neuronal impluse activity. At present we can only speculate whether these alterations in dopamine transmission play a role in the etiopathology of schizophrenia or reflect a biological response to the disease. Mass spectrometric studies are now under way in an effort to demonstrate concentrations of monopropionylcadaverine in the relevant brain regions.

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