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Potassium markedly potentiates the effect of veratridine on dopamine release from rat superfused striatal ribbons

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Abstract—The effects of veratridine-induced depolarization on [^{3}H]dopamine ([^{3}H]DA) release in the presence of a physiological (5 mM) or a depolarizing (25 mM) concentration of K^+ were studied in-vitro in rat superfused striatal ribbons. A combination of the two depolarizing agents induced a marked potentiation in the overflow of [^{3}H]DA, giving an overall 3- to 5-fold increase in veratridine activity. This potentiation was completely antagonized by tetrodotoxin (100 nM). These studies indicated that K^+-induced depolarization can increase the potency of veratridine in releasing dopamine from terminals.

The classical sequence of events that occur at central nerve endings—depolarization, increase in Ca^{2+} permeability, Ca^{2+} entry and transmitter release—has been well documented (Blaustein 1975). It has been demonstrated, by experiments with isolated brain tissue (synaptosomes or slices), that dopamine release from central neurons can be evoked by electrical stimulation and by K^+ or veratridine addition (Farnebo & Hamberger 1971; Bustos & Roth 1972; Mulder et al 1975; Westfall et al 1976; Plotsky et al 1977; Starke et al 1978; Glowinski et al 1980).

Herdon et al (1985) have reported that the K^+-evoked release of either endogenous or [^{3}H]dopamine ([^{3}H]DA) can be divided into two phases: a slight stimulation of release, which occurs at K^+ concentrations up to 25 mM (1st phase), followed by a much greater stimulatory effect, observed with K^+ concentrations of about 50 mM (2nd phase). This paper reports the effects, observed in rat striatal ribbons, of veratridine-induced depolarization on [^{3}H]DA release in the presence of a physiological (5 mM) or a depolarizing (25 mM) concentration of K^+.

Materials and methods

Male Sprague-Dawley rats (200–250 g, Charles River, Saint-Aubin, les Elbeuf, France), housed in groups of five per cage, were maintained in a controlled environment with a 12 h light-dark cycle for at least 10 days. Food and water were freely available.

Rats were decapitated and the striata were dissected out and cooled in a modified Krebs medium (Hétier et al 1988) of composition (mM): NaCl 118, KCl 5, $NaHCO_3$ 25, NaH_2PO_4 1, $MgSO_4$ 1·2, $CaCl_2$ 1·9, glucose 11·1, ascorbic acid 0·11 and pargyline 10 μM. The tissue was cut into ribbons (0·3 × 0·3 mm) with a McIlwain chopper and was immediately incubated in 5 mL modified Krebs solution (see above) containing 0·1 μM [^{3}H]DA (40–50 Ci mmol^{-1}, Amersham) for 15 min at 37°C. The tissue was then rinsed, and samples containing approximately 15 mg tissue were transferred to superfusion chambers consisting of Millipore filters (0·45 μm). After 30 min of superfusion at 0·4 mL min^{-1}, 2-min samples were collected directly into vials and the amount of radioactivity was determined by liquid scintillation spectrometry. Depolarization-induced increase of [^{3}H]DA release was accomplished by superfusion, for 1 min, with a medium containing either 25 mM KCl or veratridine at various concentrations. The depolarization was effected 8 min (4 fractions) after the beginning of the collection period. A total of 9 fractions was collected. The radioactivity remaining in the filter at the end of the superfusion period was measured, and radioactivity was expressed as a percentage of the total radioactivity present in the ribbons at the beginning of each fraction. The fractional rate of [^{3}H]DA release was reported for 2-min fractions (see Fig. 2). Moreover, the total overflow (defined as the efflux above baseline that occurred as the result of the stimulation) was expressed as the percentage of total radioactivity present in the slices just before the depolarization (see Figs 1, 3). Values expressed represent mean ± s.e.m. Data were analysed by Student's *t*-test. Significance was defined as $P < 0.05$. Veratridine and tetrodotoxin were purchased from Sigma Chemicals (La Verpilliere, France). MK 801 was purchased from Research Biochemicals Inc., Natick, MA.

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Results

The percentage of [^{3}H]DA released in the first of the 2-min samples collected averaged 0·684 ± 0·02 (n = 50). Approximately 85% of the radioactivity from base-line, or from K^+ (25 mM)- or veratridine (5 μM)-stimulated fractions was recovered by alumina chromatography. Since the metabolism of dopamine had

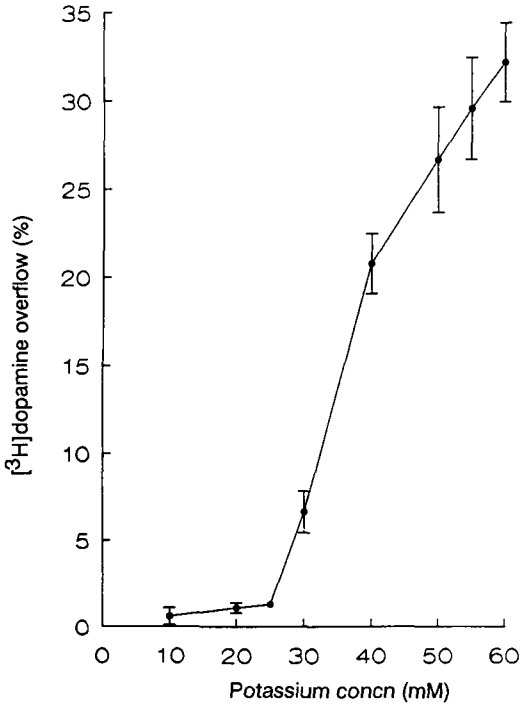

FIG. 1. Overflow of [^{3}H]DA in response to different concentrations of potassium. Results are expressed as the mean ± s.e.m. of 3 to 10 determinations. For each concentration, the total overflow was calculated as the difference between (1) the percent release induced by depolarization in all the fractions combined and (2) the percent release in the combined fractions obtained with 5 mM K^+ (baseline value).

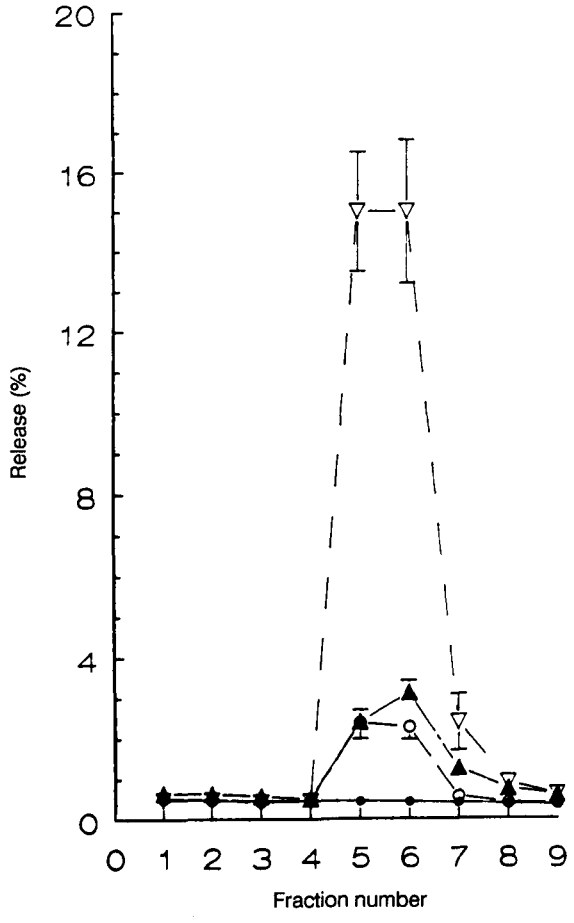

FIG. 2. Effects of veratridine (5 μM, ▲), K^+ (KCl 25 mM, ○) and their combination (▽) on [^{3}H]DA release in striatal ribbons. Results are expressed as the mean ± s.e.m. of 3 determinations. The amounts of [^{3}H]DA released for each depolarizing agent and their combination were significantly different from the control (●) values in the 5th and 6th fractions ($P < 0.05$; Student's t-test).

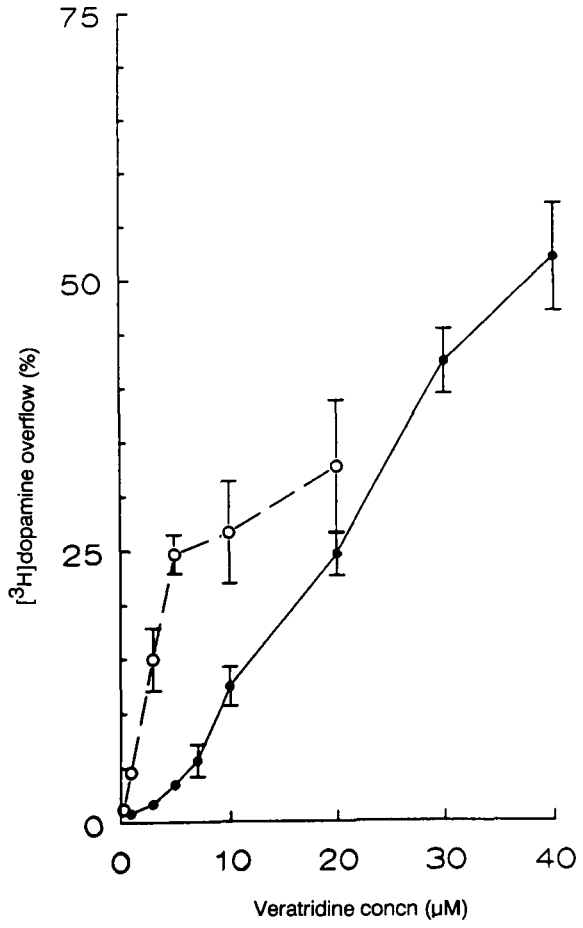

FIG. 3. Comparison of the effects of normal ($K^+ = 5$ mM, ●) and depolarizing ($K^+ = 25$ mM, ○) Krebs media on veratridine-induced [^{3}H]DA release in striatal ribbons. [^{3}H]DA overflow from the 6th to the 9th fraction of superfusion was calculated as reported in Fig. 1. Results are expressed as the mean ± s.e.m. in triplicate from 7 experiments.

been prevented, the major part of [^{3}H]catechols retained on the alumina was considered to be [^{3}H]DA.

As Fig. 1 shows, increasing the concentration of K^+ from 5 to 60 mM had a biphasic effect on the total overflow of [^{3}H]DA. The first phase, observed with concentrations of K^+ up to 25 mM, corresponded to a small increase in [^{3}H]DA release. A much greater stimulation was observed with K^+ concentrations between 25 and 60 mM. This result is in good agreement with those previously reported by Herdon et al (1985). The dose-response curve for the veratridine-evoked release of [^{3}H]DA was linear (see Fig. 3).

Fig. 2 shows that raising the K^+ concentration from 5 to 25 mM increased the fractional rate of [^{3}H]DA outflow approximately 4- to 5-fold. A release of the same magnitude was generally observed with 5 μM veratridine. Under our experimental conditions, the veratridine-evoked release of [^{3}H]DA was antagonized by 100 nM tetrodotoxin but the release evoked by K^+ (25 or 55 mM) was not (data not shown). A combination of veratridine (5 μM) and K^+ (25 mM) greatly potentiated the [^{3}H]DA overflow (Fig. 2). The effect of various concentrations of veratridine on [^{3}H]DA overflow in the presence of K^+ at either a physiological (5 mM) or a depolarizing (25 mM) concentration was also investigated. We observed a shift to the left of the dose-response curve for veratridine in the presence of 25 mM K^+, indicating that the activity of veratridine had increased 3- to 5-fold (Fig. 3). Moreover, when veratridine, at a concentration of 0·3 μM which was itself inactive, was combined with 25 mM K^+, the overflow of [^{3}H]DA significantly increased (50%). In the presence of tetrodotoxin (100 nM), the potentiation observed with veratridine (3 μM) and K^+ (25 mM) was completely reversed (Table 1): the value for [^{3}H]DA release was similar to that observed with 25 mM K^+ alone (see also Table 2). Substitution of the normal superfusion medium by one without added Ca^{2+} and containing 1·9 mM EGTA reduced the basal release of [^{3}H]DA by 30–40% (data not shown), the K^+ (25 mM)-evoked release by 80% and the veratridine (5 μM)-evoked release by about 55% (Table 2). The potentiation observed with a combination of veratridine and K^+ was reduced by 65% in the absence of Ca^{2+} (Table 2).

Table. 1. Effects of tetrodotoxin and MK 801 on the release of [^{3}H]DA evoked by a combination of K^+ (25 mM) and veratridine (3 μM).

	Control	Tetrodotoxin (100 nM)	MK 801 (1 μM)
% Overflow	25·4 ± 1·6	2·8 ± 0·2	24·6 ± 5·0

The release is expressed as the percentage of [^{3}H]DA overflow from the 6th to the 9th fraction of superfusion. Tetrodotoxin and MK 801 were added 4 min before the depolarizing agents. Results are expressed as the mean ± s.e.m. of n = 3 to 6 determinations.

Table 2. Effects of calcium removal on evoked [^{3}H]DA release.

	K^+ (25 mM)	Veratridine (5 μM)	Combination
Control	2·9 ± 0·2	5·7 ± 0·3	30·3 ± 1·5
$-Ca^{2+}$	0·6 ± 0·1	2·6 ± 0·5	11·0 ± 0·3

The release is expressed as the percent of [^{3}H]DA overflow from the 6th to the 9th fraction of superfusion. When calcium was omitted, 1·9 mM EGTA was added at the beginning of the superfusion. Results are expressed as mean ± s.e.m. of 3 to 6 determinations.

Finally, 1 μM MK 801 did not affect the increase in [^{3}H]DA release (Table 1).

Discussion

Previous papers have reported the effects of depolarizing agents on [^{3}H]DA release in in-vitro superfused tissues (see Glowinski et al 1980; Chesselet 1984). In this study, K^+ and veratridine at various concentrations were used to depolarize membranes of rat striatum in order to study the effects on [^{3}H]DA release. Depolarization induced by K^+ at increasing concentrations yielded a biphasic dose-response curve, confirming the results of Herdon et al (1985). However, when depolarization was induced by veratridine, the response was linear. Twenty-five mM K^+ and 5 μM veratridine each increased the fractional rate of [^{3}H]DA release approximately 4- to 5-fold. When both depolarizing agents were introduced simultaneously, a striking potentiation of this effect was observed (approx. 30 times the basal value). The comparison of the effects of veratridine in the presence of two different concentrations of K^+, 5 and 25 mM, showed a shift to the left of the dose-response curve for veratridine in the presence of a 25 mM K^+ concentration, indicating that veratridine activity had increased 3- to 5-fold.

The present results indicate that K^+-induced depolarization increases the potency of veratridine to release dopamine from nerve terminals. The mechanism underlying the K^+-induced release of a neurotransmitter generally requires extracellular Ca^{2+}, whereas veratridine can induce neurotransmitter release by mobilizing Ca^{2+} from intracellular stores (Cunningham & Neal 1981; Herdon et al 1987). Under our experimental conditions, the mechanism underlying the K^+-evoked release of [^{3}H]DA was more Ca^{2+} dependent than that underlying the veratridine-evoked response. The Ca^{2+} dependency of the combination of 5 μM veratridine and 25 mM K^+ was only slightly higher than that of veratridine alone, suggesting that K^+ addition does not modify markedly the availability of Ca^{2+} for veratridine activity. Similarly, it is unlikely that K^+ increases the ability of veratridine to mobilize intracellular Ca^{2+}. However, the increase in veratridine-evoked [^{3}H]DA in the presence of 25 mM K^+ could be the consequence of a specific voltage-dependent increase in Ca^{2+} conductance (gCa^{2+}).

Activation of *N*-methyl-D-aspartate (NMDA) receptors contributes to the presynaptic regulation of dopamine. In rat striatal slices, in the absence of magnesium, NMDA stimulates the release of dopamine (Ransom & Deschenes 1989). In cortex slices, when the blocking effect of Mg^{2+} is counteracted by a continuous depolarization with K^+ or veratridine, NMDA stimulates noradrenaline release (Fink et al 1990). Thus, it could be proposed that the excitatory effect of glutamate (released from glutamate terminals) is potentiated at the level of dopaminergic terminals by K^+ and veratridine. However, since MK 801, a known NMDA antagonist (1 μM), did not antagonize the increase in [^{3}H]DA release induced by the combination of the two depolarizing agents, endogenous glutamate released in response to the depolarizing stimuli is apparently not involved in the phenomenon we observed.

As the Na^+ channel opening is voltage-dependent, it is tempting to speculate that, in the presence of 25 mM K^+, this channel assumes a molecular conformation which favours the access of veratridine to its binding sites. However, the exact mechanisms involved in the effects reported in the present study remain to be determined.

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