J. Pharm. Pharmacol. 1991, 43: 798–801
Received March 13, 1991



# Differential Effects of Potassium Channel Blockers on Dopamine Release from Rat Striatal Slices

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**Abstract**—The effects of different potassium channel blockers on tritiated dopamine ([$^3$H]DA) release were investigated in rat striatal slices in the presence of pargyline and nomifensine (10 μM each). 4-Aminopyridine (4-AP; 10 and 30 μM) and 3,4-diaminopyridine (3,4-DAP; 30 μM) markedly increased the basal tritium outflow, whereas tetraethylammonium (TEA; 100–1000 μM) was without effect. The facilitating effect of 4-AP (10 μM) on spontaneous release was $Ca^{2+}$- and $K^+$-dependent. Moreover, the 4-AP-induced increase in spontaneous release was abolished in the presence of tetrodotoxin, indicating that voltage-dependent $Na^+$ channels were involved in the release mechanism. 4-AP (10 and 30 μM) induced a dose-dependent decrease in $K^+$-evoked [$^3$H]DA release. This effect was confirmed with 3,4-DAP (30 μM). When striatal slices were depolarized with veratridine (5 μM), these two aminopyridines increased the evoked release of [$^3$H]DA. TEA increased both $K^+$- and veratridine-evoked [$^3$H]DA release. These biochemical results are consistent with electrophysiological differences between the mechanism of action of aminopyridines and that of TEA.

It has now been clearly demonstrated that aminopyridines and tetraethylammonium (TEA) are selective blockers of $K^+$ channels in excitable membranes (for reviews, see Glover 1982; Cook 1988). At various synaptic junctions, 4-aminopyridine (4-AP) and TEA are known to potentiate the release of neurotransmitter evoked by nerve stimulation (Lundh & Thesleff 1977; Lamarca & Collier 1983; Kumamoto & Kuba 1985). A recent electrophysiological investigation (Tepper et al 1986) has suggested that, in urethane-anaesthetized rats, TEA and 4-AP decrease neuronal excitability by increasing the release of dopamine (DA) from striatal nerve terminals. The present experiments were carried out in an attempt to demonstrate the facilitation of [$^3$H]DA release by $K^+$ channel blockers in rat striatal slices in-vitro. Depolarization of nerve cell membranes can be induced in-vitro by reducing the concentration gradient of potassium between the intracellular and the extracellular space with hypertonic KCl, or alternatively with veratridine, which elicits depolarization by increasing the permeability of the cell membrane to $Na^+$ ions (Shanes 1958). Both depolarizing agents stimulate calcium uptake by synaptosomes (Blaustein 1975) and provoke exocytosis from sympathetic nerve terminals (Thoa et al 1975). We thus studied the effects of 4-AP, 3,4-diaminopyridine (3,4-DAP) and TEA on spontaneous or evoked [$^3$H]DA release using $K^+$ or veratridine as depolarizing agents. In this study, newly uptaken DA from striatal terminals was used, since Herdon & Nahorski (1987) and Hétier et al (1988) have recently reported that, despite some differences between the $K^+$-evoked release of [$^3$H]DA and release of endogenous DA, their regulation appears to be similar.

## Materials and Methods

Male Sprague-Dawley rats (CD, C.O.B.S. from Charles River, Saint-Aubin-lès-Elbeuf, France), 190–230 g, were used. Striatal slices of 0·3 mm thickness were obtained with a McIlwain tissue chopper and immediately incubated for 15 min with [$^3$H]DA (Amersham, 40–50 Ci mmol$^{-1}$) at a final concentration of 0·1 μM in a modified Krebs medium (composition in 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; pargyline 0·01) continuously gassed with 95% $O_2$–5% $CO_2$. The slices were then rinsed with Krebs medium, deposited in superfusion chambers (cylindrical plexiglass, 10 mm diameter, vol 0·2 mL; 10 mg original tissue in each chamber) and superfused at a rate of 0·8 mL min$^{-1}$ with Krebs medium containing nomifensine (10 μM), a potent DA uptake inhibitor (Hunt et al 1974). The superfusate was collected over several 5 min periods. Depolarizing agents, either 25 mM KCl (the $Na^+$ concentration was lowered correspondingly to maintain isotonic conditions) or veratridine (5 μM) was added to the superfusion medium for one min at the 60th (S1) and 85th (S2) min after the beginning of the superfusion. For each depolarization, the major part (90–95%) of the evoked release of radioactivity was recovered in the S1 or S2 fraction. Potassium channel blockers were added 15 min before S2 and maintained until the end of the experiment. In control samples, both S1 and S2 were carried out in the absence of drugs. Tritium radioactivity was measured in the superfusates and in tissue slices by liquid scintillation counting. Spontaneous and evoked release rates were calculated for each 5 min fraction as a percentage of the total tissue radioactivity present at the beginning of each fraction. The effects of the drugs on the depolarization-induced [$^3$H]DA release were evaluated by calculating the ratio of the overflow evoked by S2 to that evoked by S1 (S2/S1). In the same way, the effects on basal tritium outflow were expressed as the ratios of the fractional rate of tritium release immediately before S2 to that in the fraction before S1 (Sp2/Sp1). The results are presented as the means ± s.e.m., n is the number of experimental samples. Statistical difference between the means was analysed using Student's *t*-test.

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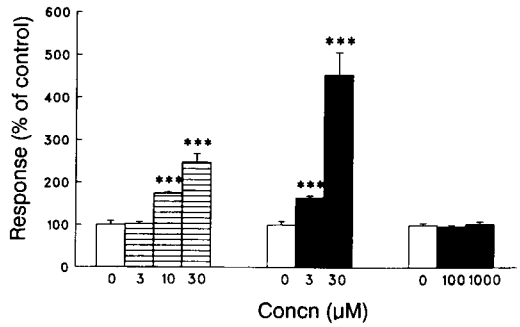


FIG. 1. Effects of 4-AP (3, 10, 30 μM) (left), 3,4-DAP (3, 30 μM) (centre) and TEA (100, 1000 μM) (right) on spontaneous release of [$^3$H]DA. Results are Sp2/Sp1 ratios expressed as % of respective control values. Each data point represents the mean ± s.e.m. of 4 to 6 determinations. *** $P < 0.001$.

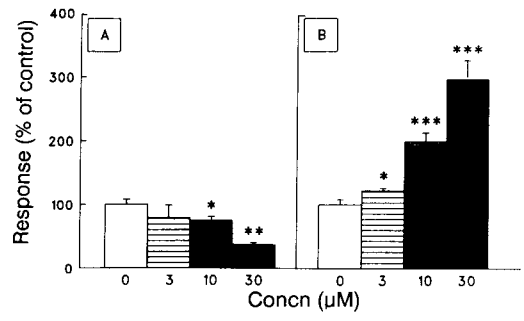


FIG. 3. Effects of 4-AP on evoked [$^3$H]DA release. A: $K^+$ depolarization (25 mM). B: Veratridine depolarization (5 μM). Results are S2/S1 ratios expressed as % of respective control values. Each data point represents the mean ± s.e.m. of 4 to 6 determinations. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

## Results

*Control results*

The percent release of [$^3$H]DA in the 5 min samples before the first depolarization averaged 1·039 ± 0·018 (n = 197). During the first period of stimulation, potassium (25 mM) depolarization induced an outflow of 3·302 ± 0·135 (n = 135) whilst veratridine (5 μM) induced an outflow of 3·914 ± 0·301 (n = 62). Control S2/S1 ratios were 0·565 ± 0·017 (n = 71; $K^+$ depolarization) and 0·395 ± 0·023 (n = 30; veratridine depolarization). Control Sp2/Sp1 ratios were 0·820 ± 0·011 (n = 71; $K^+$ depolarization) and 0·823 ± 0·019 (n = 30; veratridine depolarization). Approximately 86–87% of the radioactivity in the basal, $K^+$ or veratridine-stimulated fractions was recovered as [$^3$H]catechols from alumina chromatography. Since dopamine metabolism was prevented, the major part of [$^3$H]catechols retained on alumina could be assumed to be [$^3$H]DA. Under our experimental conditions, this $K^+$- and veratridine-induced release of labelled dopamine was found to be dependent on the presence of $Ca^{2+}$ ions (data not shown). Moreover, the S2/S1 ratio when using $K^+$ as a depolarizing agent was not modified with tetrodotoxin as previously reported (Hétier et al 1988).

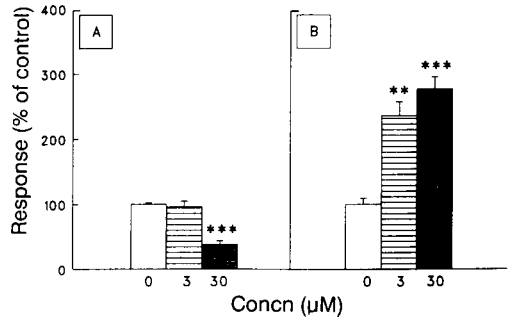


FIG. 4. Effects of 3,4-DAP on evoked [$^3$H]DA release. A: $K^+$ depolarization (25 mM). B: Veratridine depolarization (5 μM). Results are S2/S1 ratios expressed as % of respective control values. Each data point represents the mean ± s.e.m. of 4 to 6 determinations. ** $P < 0.01$; *** $P < 0.001$.

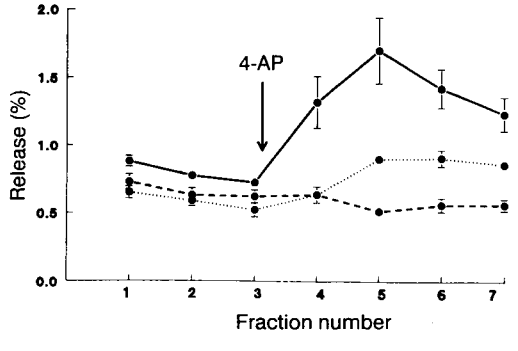


FIG. 2. Effects of calcium-free (- - - -) and low (1 mM) $K^+$ (·····) on 4-AP-induced [$^3$H]DA release (——). Slices previously labelled with [$^3$H]DA were superfused with different media. At t = 40 min, 5 min fractions were collected; 4-AP (20 μM) was added at t = 55 min and maintained until the end of the superfusion. Each result is the mean ± s.e.m. of 4 individual determinations.

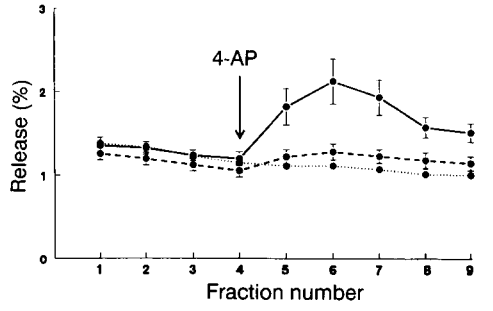


FIG. 5. Effects of tetrodotoxin (100 nM) on 4-AP-induced increase in spontaneous [$^3$H]DA release. 4-AP, 20 μM (arrow) was introduced two fractions after tetrodotoxin and maintained until the end of the superfusion. Tetrodotoxin 100 μM (·····), 4-AP 20 μM (——) and the combination (- - - -). Each datum represents the mean ± s.e.m. of 3 to 4 determinations.

*Effects of 4-AP and 3,4-DAP on [$^3$H]DA release*

As shown in Fig. 1, 4-AP and 3,4-DAP induced a marked increase in basal tritium outflow (Sp2/Sp1 ratio). When calcium was removed from the superfusion medium (1·9 mM $MgCl_2$), the increase in spontaneous [$^3$H]DA release induced by 4-AP was completely abolished (Fig. 2). In the presence of low (1 mM) potassium levels, 4-AP produced a modest increase in [$^3$H]DA release (Fig. 2). Complete removal of $K^+$ ions from the superfusion medium induced a more pronounced increase (5 times) in spontaneous [$^3$H]DA release. In

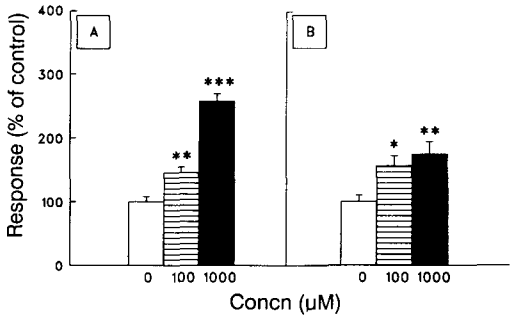


FIG. 6. Effects of TEA on evoked [$^3$H]DA release. A: $K^+$ depolarization (25 mM). B: Veratridine depolarization (5 μM). Results are S2/S1 ratios expressed as % of respective control values. Each datum represents the mean ± s.e.m. of 4 to 6 determinations. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

the absence of $K^+$, 4-AP (10 μM) was not able to increase the spontaneous outflow of [$^3$H]DA to a significant extent (data not shown).

When added to the superfusion medium, 4-AP induced a dose-dependent decrease of $K^+$-evoked [$^3$H]DA release (Fig. 3). Similar results were obtained with 3,4-DAP (Fig. 4).

As shown in Fig. 3, veratridine-evoked [$^3$H]DA release was increased by 4-AP in a dose-dependent manner. The effects observed with 4-AP were also observed with 3,4-DAP in slices (Fig. 4).

In the presence of 100 nM tetrodotoxin (TTX), the veratridine-induced increase in [$^3$H]DA release was completely abolished (data not shown). When added to the superfusion medium, TTX (100 nM) markedly antagonized the increase in [$^3$H]DA release induced by 4-AP (Fig. 5).

*Effects of TEA on [$^3$H]DA release*

TEA (0·1, 1 mM) was devoid of effect on spontaneous [$^3$H]DA release (Fig. 1), but markedly increased both the $K^+$- and the veratridine-evoked [$^3$H]DA release (Fig. 6).

## Discussion

This paper reports the effects of different blockers of voltage-sensitive $K^+$ channels on the spontaneous and evoked release of newly-uptaken [$^3$H]DA in rat striatal slices. Since voltage-sensitive potassium channels participate in nerve terminal excitability, the application of potassium channel blockers would be expected to increase the release of dopamine from these terminals. The electrophysiological results reported by Tepper et al (1986) suggest that 4-AP and TEA cause the release of endogenous dopamine from striatal terminals. The main objective of the present study was to determine directly whether potassium channel blockers modulate dopamine release in-vitro.

In 5 mM KCl, 4-AP (10 and 30 μM) markedly increased the spontaneous release of [$^3$H]DA. In a calcium-free medium ($MgCl_2$ = 1·9 mM), the 4-AP-induced increase in spontaneous [$^3$H]DA release was completely abolished. In a low (1 mM) potassium medium, 4-AP exerted a less marked increase in [$^3$H]DA outflow. These results suggest that in rat striatal slices, 4-AP releases DA via a $Ca^{2+}$ and $K^+$ dependent mechanism. A possible direct effect on $Ca^{2+}$ influx cannot be ruled out, although the effect of 4-AP seems primarily due to the blockade of potassium channels (Huang et al 1989; Fredholm 1990). The facilitatory effect of 4-AP was significantly decreased in the presence of TTX, suggesting that the induction and propagation of action potentials carried by voltage-dependent $Na^+$ channels are necessary steps in the excitatory action of 4-AP. These observations are comparable with those of Dolezal & Tucek (1983), Huang et al (1989) and Tibbs et al (1989b) who studied the effect of TTX on 4-AP- or 3,4-DAP-evoked acetylcholine, noradrenaline and glutamate release. Moreover, the sensitivity of 4-AP to TTX indicates that opening of voltage-sensitive $Ca^{2+}$ channels is not the primary event in 4-AP-evoked DA release, as suggested in the case of noradrenaline (Huang et al 1989).

In the presence of 4-AP (3–30 μM), [$^3$H]DA release was less sensitive to potassium depolarization, consistent with a potassium channel blocking action of this compound, though a competitive interaction between 4-AP and $K^+$ cannot be ruled out. Our results are in good agreement with those of Dolezal & Tucek (1983) and Tibbs et al (1989a), who reported that 4-AP decreased the $K^+$-evoked release of acetylcholine or glutamate from cortical slices or synaptosomes, respectively. By contrast, when [$^3$H]DA release was evoked by the sodium channel activator veratridine (5 μM), 4-AP (3-30 μM) increased the overflow in a dose-dependent manner, indicating a synergy between these two different mechanisms. The effects of 3,4-DAP (3, 30 μM) were essentially the same as those of 4-AP.

The facilitating effects of aminopyridines on striatal dopamine release are similar to those observed in the peripheral nervous system at micromolar concentrations (Lundh 1978; Kumamoto & Kuba 1985).

In contrast to the results obtained with 4-AP and 3,4-DAP, TEA (0·1 and 1 mM), which interacts with a different population of potassium channels (Thompson 1977), had no effect on spontaneous [$^3$H]DA release, but markedly increased $K^+$-evoked [$^3$H]DA release. However, like 4-AP, it did potentiate veratridine-evoked [$^3$H]DA release. It is noteworthy that the concentrations which increase the release of [$^3$H]DA are very close to those used by Kirpekar et al (1972). The finding that spontaneous release is not affected by TEA could be explained in two ways. Firstly, that the delayed channels which it blocks (Ikv) are not active under resting conditions (Cook 1988) and secondly, that these channels are remote from the areas of the synapse at which depolarization-evoked exocytosis occurs. Since TEA enhances [$^3$H]DA release evoked by both potassium and veratridine, it is possible that the TEA-sensitive channels may regulate the action potential invasion of the nerve terminals. It is noteworthy that micromolar concentrations (as opposed to millimolar concentrations) of 4-AP do not prolong the neuronal action potential (Burley & Jacobs 1981), whilst TEA causes a very large increase in the action potential duration (Glover 1982). A similar segregation of function has been proposed on the basis of release experiments for the different types of voltage-sensitive calcium channel, where the N channel seems to be involved in exocytosis and the T channel in nerve terminal invasion (Miller 1987).

The results reported in the present study are somewhat different to those of Wemer et al (1981), who showed that 4-AP (0.03–3 mM) can enhance $K^+$ (13 mM)-induced [$^3$H]-

noradrenaline release from cortical slices. However, those authors reported that the enhancing effect of 4-AP was greatly reduced when 30 mM $K^+$ was used. Moreover, Burley & Jacobs (1981) have demonstrated in the peripheral nervous system that, whilst low concentrations of aminopyridines can enhance neurotransmitter release without broadening the neuronal action potential, higher concentrations will produce both effects. The comparison of the effects observed by Wemer et al (1981) with our data for TEA may be more appropriate.

In conclusion, we have shown that both aminopyridines and TEA can modulate the evoked release of [$^3$H]DA, consistent with the hypothesis that potassium channels play a role in neurotransmitter release. However, major differences were observed between the effects of the aminopyridines and those of TEA, according to the conditions under which [$^3$H]DA release was measured. This may reflect differences in the specificity of these blockers for the various subtypes of potassium channel which have been observed in electrophysiological experiments (Ikv being most sensitive to TEA and Ia (A-current) to aminopyridines). Recent reports concerning the effect of aminopyridine on the activation of protein kinase C (Huang et al 1989; Heemskerk et al 1990) support the hypothesis that blockade of Ia current may lead to a facilitation of neurotransmitter release. Moreover, from our own results, we could speculate that the aminopyridine-sensitive channel is tightly coupled to the release process, whereas the TEA-sensitive channel controls the action potential invasion of the nerve terminals.

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