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Ouabain-induced Increase in Dopamine Release from Mouse Striatal Slices is Antagonized by Riluzole

A. BOIREAU, M. MEUNIER AND A. IMPERATO

Rhône-Poulenc Rorer S. A., Centre de Recherche de Vitry-Alfortville, 13, quai Jules Guesde BP 14, 94403 Vitry-sur-Seine Cedex, France

Abstract

We have examined the effects of riluzole, a neuroprotective drug which stabilizes voltage-dependent sodium channels in their inactivated state and inhibits the release of glutamate in-vivo and in-vitro, on the release of newly taken up [^{3}H]dopamine induced by ouabain, a potent and selective inhibitor of Na^+/K^+-ATPase in mouse striatal slices in-vitro.

Riluzole potently (IC50 (concentration resulting in 50% inhibition) = 0·9 ± 0·3 μM) and dose-dependently antagonized ouabain-stimulated [^{3}H]dopamine release, the effect being observed at low concentrations. Tetrodotoxin (1 μM) and nomifensine (10 μM) also abolished ouabain-induced [^{3}H]dopamine release. Blockade of glutamate receptors with dizocilpine (1 μM) and 6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (YM-90K; 10 μM), alone or in combination, was without effect. Incubation of striatal slices with 50 μM La^{3+}, which blocks voltage-dependent calcium channels, did not inhibit [^{3}H]dopamine release induced by ouabain.

The potent effects of riluzole observed in this model are probably related to its ability to block voltage-dependent sodium channels. The consequences of this activity are critically discussed in relation to the protective action of riluzole previously reported in various models of Parkinson's disease and other neurodegenerative disorders.

It has been suggested that impairment of energy homeostasis in neurones is involved in neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and Huntington's disease (Beal 1992). Interestingly, the sodium/potassium-ATPase (Na^+/K^+-ATPase) uses at least 40% of the ATP synthesized by the brain (Astrup et al 1981; Lees 1991). It has been suggested that in neuropathological states disruption of ATP synthesis might induce a major deficiency in Na^+/K^+-ATPase activity (Lees 1993) and this might represent a key step in the cascade of events that follows impairment of cellular energy metabolism. Ouabain induces neurotoxicity when injected into different cerebral structures by inhibiting Na^+/K^+-ATPase (Lees et al 1990; Lees & Leong 1995) and in-vitro ouabain increases the release of neurotransmitters, including dopamine (Vizi 1972, 1978). Ouabain also increases dopamine release in-vivo (Sirinathsinghji et al 1988; Hurd & Ungersted 1989; Fairbrother et al 1990). It is noteworthy that the consequences of the deficiency in ATP production in Parkinsonism might be greatly amplified by the inability of the Na^+/K^+-ATPase pump to maintain Na^+ homeostasis (Johnson et al 1992).

Correspondence: A. Boireau, Magendie building, Rhône-Poulenc Rorer S. A., Centre de Recherche de Vitry-Alfortville, 13, quai Jules Guesde BP 14, 94403 Vitry-sur-Seine Cedex, France.

Riluzole (2-amino-6-trifluoromethoxybenzothiazole) is a neuroprotective drug the mechanism of action of which has recently been reviewed (Doble 1996). Riluzole does not bind to any known glutamate-receptor subtype but interacts with excitatory amino-acid receptors (Debono et al 1993). It stabilizes voltage-dependent sodium channels in their inactivated state (Hebert et al 1994) and inhibits the release of glutamate in-vivo (Cheramy et al 1992) and in-vitro (Martin et al 1993). Riluzole has potent activity in experimental models of Parkinson's disease in both rodents and monkeys (Boireau et al 1994a, b; Benazzouz et al 1995) and has been found to antagonize seizures induced by several convulsants, including ouabain (Mizoule et al 1985), which suggests that one or more of the known mechanisms of action can prevent the impairment of neuronal function induced by the inhibitor of Na^+/K^+-ATPase.

The aim of this study was to investigate the effects of riluzole in a ouabain-sensitive neuronal

model. For this purpose, we characterized pharmacologically the effect of ouabain on the release of newly taken up [^{3}H]dopamine from mouse striatal slices. This study reports the effects of tetrodotoxin, a sodium-channel blocker, dizocilpine (MK-801), an *N*-methyl-D-aspartate (NMDA) receptor blocker, 6-(-1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (YM-90K), a blocker of α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) receptors (Okada et al 1996), nomifensine, a known dopamine-uptake blocker, and lanthanum (La^{3+}), which blocks voltage-dependent calcium channels (Bernath 1992). Our results show that riluzole antagonizes ouabain-induced [^{3}H]dopamine release in this in-vitro model.

Materials and Methods

Male mice (Charles River, France), 20–35 g, were housed ten to a cage in a controlled environment with a 12-h light–dark cycle. Food and water were freely available. The in-vitro release of dopamine was studied as described elsewhere (Boireau et al 1993) with slight modifications. Briefly, mouse striata were sliced into ribbons (0·3 × 0·3 mm) with a McIlwain tissue chopper and incubated for 15 min at 37°C in an oxygenated (95% O_2, 5% CO_2) physiological medium comprising: NaCl 118 mM, KCl 5 mM, $NaHCO_3$ 25 mM, NaH_2PO_4 1 mM, $MgSO_4$ 1·2 mM, $CaCl_2$ 1·9 mM, glucose 11·1 mM, ascorbic acid 0·1 mM, pargyline 10 μM and [^{3}H]dopamine (1140 GBq mmol^{-1}, New England Nuclear) 0·05 μM. The tissue was rinsed and 1-mL samples containing approximately 10 mg tissue were transferred to superfusion chambers consisting of Millipore filters (Millex HA; 0·45 μm). After 30 min superfusion at 0·4 mL min^{-1}, 4-min fractions were collected directly into vials and the amount of radioactivity was determined by liquid scintillation spectrometry. Ouabain was generally added in fractions 4 to 6 (12 min) and riluzole, dizocilpine, YM-90K or tetrodotoxin were added 4 min before ouabain and maintained throughout the superfusion (La^{3+} and nomifensine were added 8 min and 12 min, respectively, before ouabain). Overall, 10 fractions from superfusion were collected. The radioactivity remaining on the filter at the end of the superfusion was measured. Radioactivity was expressed as a percentage of the total radioactivity present in the slices at the beginning of each fraction. The results are given as percent release for each fraction; the seventh fraction was usually that of maximum response. The concentration of riluzole inhibiting by 50% the effects of ouabain on the overflow from the seventh fraction (IC50 value) was calculated by computer-assisted iterative non-linear regression analysis with the Enzfitter software package; n refers to the number of experiments. Data from the seventh fraction were analysed by Student's *t*-test or one-way analysis of variance followed by a Dunnett multiple comparisons test. A value of $P < 0.05$ was regarded as indicative of significance. The results shown are representative of at least two experiments, except for riluzole for which three experiments were performed. Ouabain and tetrodotoxin were purchased from Sigma (La Verpillière, France) and dizocilpine and nomifensine from RBI (Natick, MA). Riluzole and YM90K were synthesized by our chemical department.

Results

Under our experimental conditions, ouabain (5, 10 and 20 μM) induced a dose-dependent increase in [^{3}H]dopamine release (data not shown). A concentration of 10 μM was chosen for all studies reported in this paper; at this concentration the basal release of [^{3}H]dopamine was increased 2·5-fold in the seventh fraction of perfusion, with a

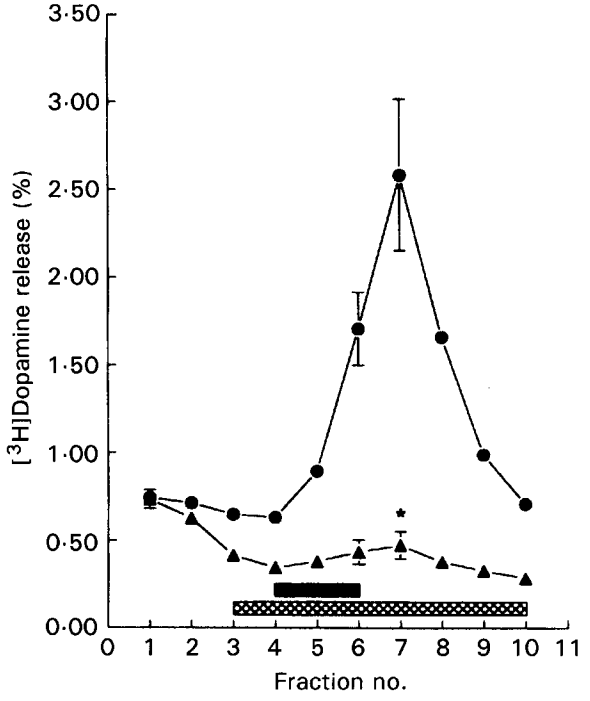

Figure 1. Effect of tetrodotoxin on the ouabain-induced release of [^{3}H]dopamine. Ouabain (10 μM; ●) was added from the 4th to the 6th fractions (black bar). Tetrodotoxin (1 μM; ▲) was added at the beginning of the 3rd fraction and maintained until the end of superfusion (grey bar). Each point represents the mean ± s.e.m. of results from three separate determinations. *$P < 0.001$, significantly different from control result (Student's *t*-test).

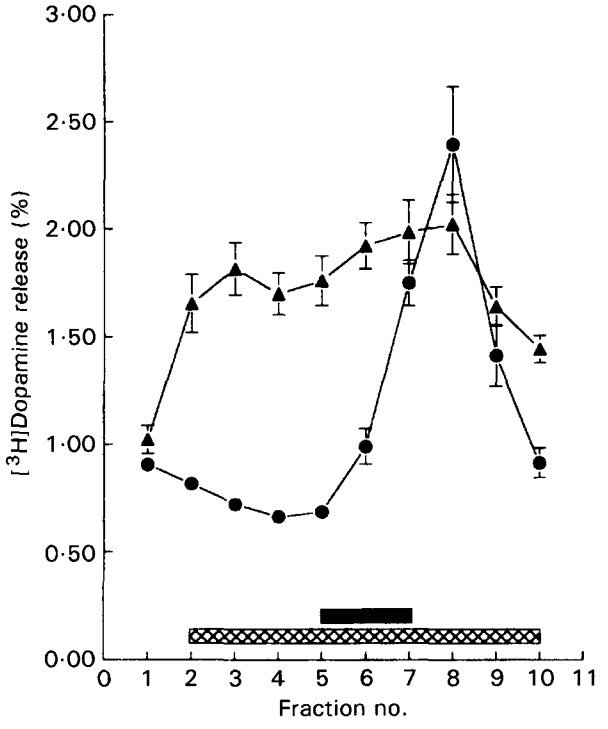

Figure 2. Effect of nomifensine on the ouabain-induced release of [^{3}H]dopamine. Ouabain (10 μM; ●) was added from the 5th to the 7th fractions (black bar). Nomifensine (10 μM; ▲) was added at the beginning of the 2nd fraction and maintained until the end of superfusion (grey bar). Each point represents the mean ± s.e.m. of results from three separate determinations.

maximum increase in [^{3}H]dopamine release observed 4 min (one fraction) after superfusion with ouabain was stopped (Figures 1–4). This slightly delayed effect was probably because the blockade of ouabain was slowly reversible (Baker et al 1969). Tetrodotoxin (1 μM) totally antagonized the effect of ouabain (Figure 1). Nomifensine (10 μM), increased the basal release of [^{3}H]dopamine, but this effect was not enhanced further with ouabain (Figure 2). In the presence of 50 μM La^{3+} the increase in [^{3}H]dopamine release induced by ouabain was still observed (data not shown). At concentrations totally blocking the glutamate- and AMPA-induced increase in dopamine release (data not shown), dizocilpine (1 μM) and YM-90K (10 μM) alone or in combination, did not modify the effect of ouabain (Figure 3). Riluzole, like tetrodotoxin, reduced the basal release of [^{3}H]dopamine (Figure 4). This effect was observed with the highest concentration of riluzole tested (10 μM). Moreover, riluzole potently (IC50 = 0·9 ± 0·3 μM; mean of three experiments in which each concentration was tested in triplicate) and dose-dependently antagonized the effect of ouabain (Figure 4).

Discussion

Our results show that ouabain dose-dependently increased the release of [^{3}H]dopamine. Under our experimental conditions, the maximum effect was observed 4 min after the end of superfusion with the Na^+/K^+-ATPase inhibitor. This delay is probably because blockade of ouabain is slowly reversible (Baker et al 1969). The releasing effect of ouabain was blocked by nomifensine, a dopamine-uptake blocker, but the blockade of voltage-dependent Ca^{2+} channels by La^{3+} was without effect. Under our experimental conditions the releasing process is far from being saturated. No additive effect was obtained in the presence of nomifensine, which emphasizes the role of the carrier in the effect of ouabain. Thus, under our experimental conditions the effect of ouabain on [^{3}H]dopamine release seems to be carrier-mediated (i.e. non-exocytotic, as reviewed recently by Levi & Raiteri 1993). Tetrodotoxin, a blocker of voltage-dependent sodium channels totally inhibited the effect of ouabain on [^{3}H]dopamine release. The sensitivity of ouabain-induced dopamine release has recently been reported under in-vivo conditions (Fairbrother et al 1990). Because we used a slice preparation,

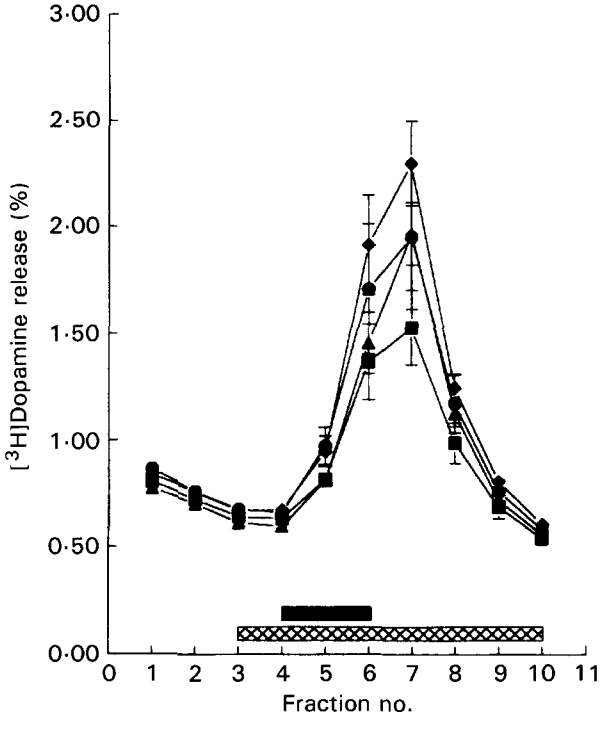

Figure 3. Effect of dizocilpine (■), YM90K (◆) or their combination (▲) on the ouabain-induced release of [^{3}H]dopamine. Ouabain (10 μM; ●) was added from the 4th to the 6th fractions (black bar). Drugs were added at the beginning of the 3rd fraction and maintained until the end of superfusion (grey bar). Each point represents the mean ± s.e.m. of results from three separate determinations.

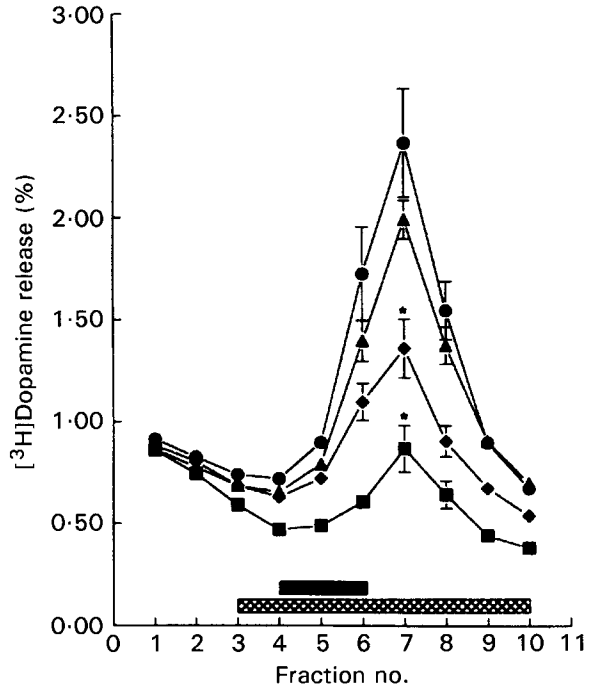

Figure 4. Dose–effect of riluzole on the ouabain-induced release of [^{3}H]dopamine. Ouabain (10 μM; ●) was added from the 4th to the 6th fractions (black bar). Riluzole (0·1 μM; (▲), 1·0 μM, (◆) or 10 μM (■)) was added at the beginning of the 3rd fraction and maintained until the end of superfusion (grey bar). Each point represents the mean ± s.e.m. of results from three independent experiments, each in triplicate. *$P < 0.01$, significantly different from control result (analysis of variance then a Dunnett's multiple comparisons test).

data obtained with tetrodotoxin might suggest that interneurones were involved in the effect we observed. This fits equally well with the proposed carrier-mediated mechanism (Levi & Raiteri 1993) involved in the releasing effect of ouabain.

The cardiac glycoside ouabain potentiates glutamate excitotoxicity in-vitro (Brines et al 1995), and the inhibition of Na^+/K^+-ATPase activity reduces the threshold for the glutamate response (Calabresi et al 1995). The immediate release of [^{3}H]dopamine induced by ouabain was not antagonized by blockade of AMPA receptors with YM-90K, by blockade of NMDA receptors with dizocilpine or by blockade of both receptors by a combination of the two antagonists, implying that under our experimental conditions the immediate release of [^{3}H]dopamine is not dependent on glutamate stimulation. It is possible that inhibition of the pump for a longer period of time is needed to enable glutamate to play a depolarizing (excitotoxic?) role on dopaminergic neurones.

More interestingly, it has been found that riluzole potently antagonizes the effect of ouabain; the effect was dose-dependent and observed at low concentrations (IC50 = 0·9 ± 0·3 μM). This concentration is identical with that observed when riluzole was tested against veratridine (5 μM) as a depolarizing agent (IC50 ≈ 1 μM; data not shown). Riluzole does not inhibit dopamine uptake, except at very high concentrations (IC50 ≈ 100 μM; T. Canton personal communication; Samuel et al 1992). Because tetrodotoxin also inhibited the response to ouabain, the antagonistic effect of riluzole in this model of dopamine release might be a result of the blockade of voltage-dependent sodium channels. It is also possible that the in-vivo anticonvulsant activity of riluzole, reported by Mizoule et al (1985) when ouabain was used as a convulsive agent, might be because of its action on sodium channels.

The data reported in this study suggest that riluzole interferes with early events that follow impairment of Na^+/K^+-ATPase, before the cascade of action that results in glutamate becoming neurotoxic. Thus, riluzole might be a drug of choice for treatment of any disease linked to a deficiency in ATP synthesis. In neuropathological diseases impaired ability to produce ATP is likely to impact on the activity of brain Na^+/K^+-ATPase (Lees 1993). In Parkinsonism impairment of energy production might be greatly amplified by the incapacity of the Na^+/K^+-ATPase pump to maintain Na^+ homoeostasis (Johnson et al 1992). A failure to maintain Na^+ homoeostasis (partly because of Na^+/K^+-ATPase deficiency) will result in excitatory amino acids becoming toxic to dopamine neurones. Thus, a key role for Na^+/K^+-ATPase in parkinsonism is a reasonable hypothesis. Whatever the mechanism involved in the development of parkinson's disease, riluzole, by reducing the energy demand in neurones, could increase their chance of survival (for review see Urenjak & Obrenovitch 1997). The data reported in this paper support previous results showing that riluzole is protective in experimental models of parkinsonism in mice (Boireau et al 1994a), rats (Boireau et al 1994b) and monkeys (Benazzouz et al 1995) and further support our initial proposal that riluzole might have clinical activity in the treatment of Parkinson's disease in man.

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