

Effect of acute hepatic encephalopathy on [^3H]dopamine release from rat cerebral cortex and striatum *in vitro*: role of Ca^{2+}

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Abstract. Hepatic encephalopathy (HE) is characterized by motor symptoms associated with disturbed functions of the dopaminergic systems, but the underlying mechanisms are not clear. A previous study from our laboratories revealed that HE, induced in rats by repeated treatment with thioacetamide, enhanced the 50 mM potassium (KCl) -stimulated release of newly loaded [^3H]dopamine in both striatal and frontal cerebral cortical slices in the presence of Ca^{2+} . In the present study we compared the effects of HE on dopamine release in striatal and frontal cerebral cortical slices and synaptosomes in the presence and absence of Ca^{2+} . HE enhanced the KCl-stimulated [^3H]dopamine release from striatal and frontal cortical synaptosomes in the presence of Ca^{2+} to the same extent as in slices prepared from the respective brain regions. In the absence of Ca^{2+} a slight reduction in dopamine release was observed in frontal cortical synaptosomes from HE rats when compared to control rats, while no effect of HE on the release was discernible in frontal cortical and striatal slices and striatal synaptosomes. We conclude that in both brain regions studied HE stimulates dopamine exocytosis triggered by Ca^{2+} influx without affecting the release mediated by means of plasma membrane transporters or exocytosis involving intraterminal Ca^{2+} .

Key words: hepatic encephalopathy, dopamine release, extracellular calcium, slices, synaptosomes, striatum, frontal cortex, potassium stimuli

INTRODUCTION

Hepatic encephalopathy (HE) is a major neuropsychiatric complication of liver disease associated with motor disturbances including clonus, rigidity and tremor, which are thought to reflect central dopaminergic dysfunction. Chronic HE patients exhibit abnormalities in the dopaminergic systems, including a decreased density of pallidal dopamine D₂ receptors (Mousseau et al. 1993) and an increased brain content of dopamine metabolites (Bergeron et al. 1989), this reflecting the increased activities of the dopamine-metabolizing enzymes monoamine oxidase (MAO) A and MAO B (Rao et al. 1993). In different experimental HE models the contents of dopamine metabolites are changed in certain brain regions (Bergeron et al. 1995). Our previous studies revealed that the thioacetamide model of HE impairs two glutamate-dependent aspects of dopamine functions in the striatum: the NMDA-dependent dopamine release *in vitro* (Borkowska et al. 1997) and the accumulation of dopamine metabolites *in vivo* (Borkowska et al. 1999).

Dopamine exhibits characteristics of classical neurotransmitters. Upon stimulation with K⁺ or other depolarizing agents a significant amount of dopamine is released from nerve endings by Ca²⁺-dependent, tetrodotoxin-sensitive exocytosis (Westerink et al. 1988, 1989), involving Ca²⁺ entry via the N, P and Q type channels (Carvalho et al. 1995). We have previously shown that thioacetamide-induced liver failure increases the release of newly loaded [³H]dopamine from striatal and frontal cortical slices in the presence of Ca²⁺ (Borkowska et al. 1997). However, some dopamine release may originate from the cytoplasmic pool by a reversed operation of dopamine carriers in plasma membranes (Raiteri et al. 1979, Hurd and Ungerstedt 1989a,b, Aliaga et al. 1995, Young and Rees 1998) and/or by exocytosis triggered by intraterminal Ca²⁺ (Oyamada et al. 1998, and references therein). In either case the release is independent of the entry of extracellular Ca²⁺. In the present study we therefore compared the effects of HE in the thioacetamide model on K⁺-evoked dopamine release in the presence and absence of Ca²⁺ in the medium. The release was measured in frontal cortical and striatal slices and synaptosomes. Synaptosomes, mainly derived from the striatum, are the fraction in which the relative contributions of the exocytotic and carrier-mediated mechanisms to the efflux of newly loaded [³H]dopamine evoked by K⁺ and other stimuli have been studied in considerable details (Bowyer et al. 1983, Okada et al. 1990, Girbe et al.

1994, Sitges et al. 1994, Carvalho et al. 1995, Soliakov and Wonnacot 1996, Crespi et al. 1997).

METHODS

Male Wistar rats weighing 180–200 g, maintained under standard conditions (light from 7 am to 7 pm, temperature 22°C, humidity 23%, with free access to standard laboratory diet and water), received at 24 h intervals three intraperitoneal injections of thioacetamide in physiological saline (300 mg/kg). The rats exhibited characteristic symptoms of HE, e.g., reduction in motor activity (Pluta and Albrecht 1984, Albrecht et al. 1994, Hilgier and Olson 1994, Borkowska et al. 1997). They were decapitated 24 h after the last injection. The excised brains were rapidly cooled on ice. The slices, 0.5 mm thick, were manually cut from the striatum (coronal section) and frontal cortex. Two middle striatal slices and the first frontal cortical slice were used for experiments. The slices were preincubated with 260 MBq/l [³H]dopamine in 2.5 ml Krebs-Ringer-HEPES medium (concentrations in mM: NaCl 126, MgSO₄ 1.29, NaH₂PO₄ 1.29, KCl 5, CaCl₂ 0.8, HEPES 15, D-glucose 10, niacinamide 4, ascorbic acid 20, NaOH 11.74, pH 7.4) for 20 min at 37°C under O₂ and then rinsed with 5 ml Krebs-Ringer-HEPES medium (without dopamine). The release experiments were carried out as previously described (Borkowska et al. 1997) with minor modifications. The freely floating shaken slices were superfused in 0.5 ml of medium at a rate of 0.25 ml/min at 37°C under O₂. The first 20-min superfusate was pooled and then 0.5 ml fractions were collected at 2 min intervals. At 28 min, the superfusion medium was changed into stimulation medium containing 50 mM KCl ("stimulated release") or left unchanged ("basal release"). After superfusion the slices were extracted overnight in 1 M NaOH. In the absence of Ca²⁺ ions the medium was supplemented with 0.5 mM ethylenediaminetetra-acetate and 0.8 mM choline chloride.

The synaptosomal fractions (S2) were prepared as described by Girbe et al. (1994) with minor modifications. The striatum and frontal cortex were homogenized in 10 volumes of cold 0.32 M sucrose with pargyline (0.1 mM) and centrifuged (1,200 g/10 min/2°C). One ml of supernatant (crude synaptosomal fraction) was preincubated with 23 MBq/l [³H]dopamine in 8.6 ml Krebs-Ringer-HEPES medium (10 min/37°C under O₂) and centrifuged (6,000 g/10 min). The synaptosomal pellet (S2) was suspended in 2.5 ml of Krebs-Ringer-HEPES solution and

superfused for 40 min at 0.25 ml/min in a Brandel multi-channel superfusion machine. At 27 min of superfusion, the medium was either changed to a stimulation medium or left unchanged. The fractions were collected at 1 min intervals. In the experiments performed in the absence of Ca^{2+} ions, CaCl_2 was omitted from the medium and MgSO_4 was increased to 1.7 mM.

The superfusate samples and extracts of the slices and S2 fractions were counted for radioactivity and the results plotted as the fractional release (Borkowska et al. 1997). The integrated area between the basal line and the curve delineating the stimulated release between 28-40 min for the slices and between 27-35 min for the S2 fractions was taken as a measure of stimulation. Statistical

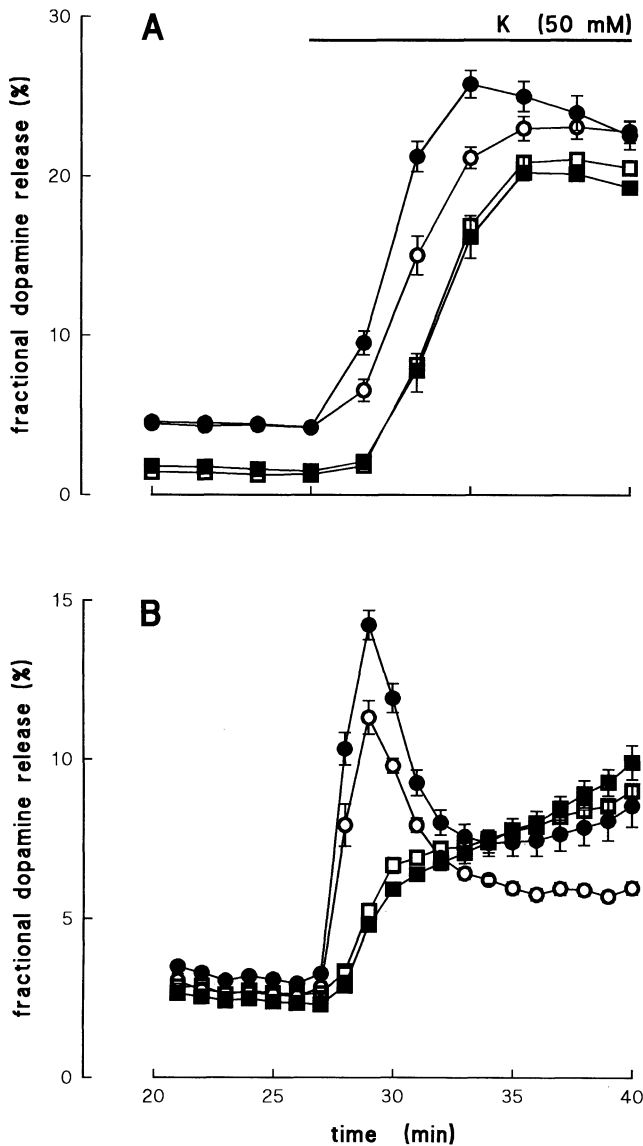


Fig. 1. K^+ (50 mM)-stimulated release of dopamine from striatal slices (A) and synaptosomes (B) from control and HE rats in the presence (open circles and close circles, respectively) and absence (open squares and closed squares, respectively) of Ca^{2+} . Results are mean values \pm SEM, for the number of experiments indicated in Table I.

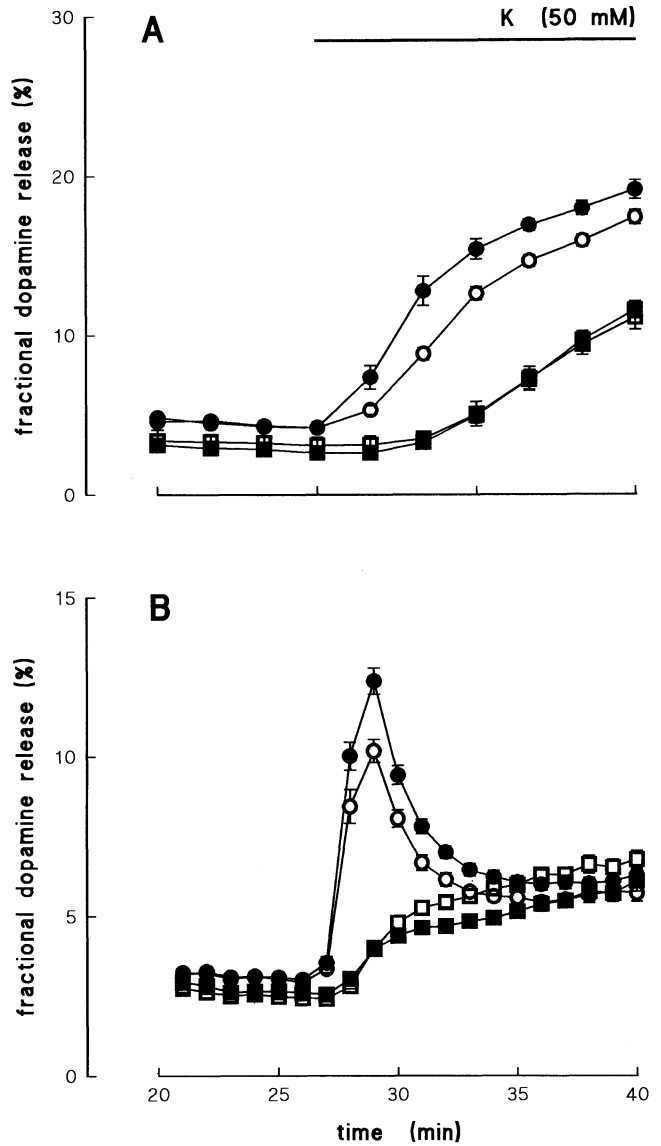


Fig. 2. K^+ (50 mM)-stimulated release of dopamine from frontal cortical slices (A) and synaptosomes (B) from control and HE rats in the presence (open circles and closed circles, respectively) and absence (open squares and closed squares, respectively) of Ca^{2+} . Results are mean values \pm SEM, for the number of experiments indicated in Table I.

evaluation of the differences was carried out with Student's *t*-test.

RESULTS

The basal release of [^3H]dopamine was not significantly changed by HE in any structure or preparation studied (Figs. 1 and 2). Fifty mM KCl evoked an increase in [^3H]dopamine release in all preparations studied, particularly in synaptosomal preparations. In cortical and striatal slices alike, the onset of stimulation was faster in Ca^{2+} -containing than in Ca^{2+} -free media, and there occurred no decline in the release during the stimulation period (Figs. 1A and 2A). In synaptosomes the release increased instantly upon addition of KCl (Figs. 1B and 2B). In the presence of Ca^{2+} , the KCl-evoked release was attenuated in a few minutes and remained thereafter at 150%-180% of the basal level, whereas in Ca^{2+} -free media the stimulatory effect of KCl persisted until the end of superfusion. In the presence of Ca^{2+} HE enhanced the stimulated release of dopamine in striatal slices and synaptosomes by 20% and 24%, respectively, and in frontal cortical slices and synaptosomes by 34% and

36%, respectively (Table I). The only significant effect of HE noted in Ca^{2+} -free medium was a 15% decrease in frontal cortical synaptosomes. The absence of Ca^{2+} attenuated the stimulated release of dopamine in all experiments except in striatal slices from control rats. This attenuation was always more pronounced in preparations derived from HE rats than in those from control rats (Table I).

DISCUSSION

There is a growing body of evidence that motor disturbances accompanying HE result to a certain degree from dopaminergic dysfunction (see Introduction). In the thioacetamide model HE enhances the release of newly loaded [^3H]dopamine from striatal and frontal cortical slices *in vitro* (Borkowska et al. 1997), but the release was measured only in the presence of Ca^{2+} . The question thus remained open whether HE affects only dopamine exocytosis associated with the influx of extracellular Ca^{2+} or also dopamine efflux by mechanisms independent of extracellular Ca^{2+} , namely carrier-mediated efflux from cytoplasm or exocytosis mobilized by

TABLE I

Effect of K^+ (50 mM) on [^3H]dopamine release from striatal and frontal cortical slices and synaptosomes from control and HE rats

Region	Ca^{2+}	Preparation	Stimulated release (integrated area)		
			Control	HE	% change
Striatum	+	slices	157.4 \pm 6.4 (15)	188.1 \pm 7.3 (14)*	+20 ^a
	-		144 \pm 10.5 (6)	136.7 \pm 9.2 (8)	NS
		% change	NS	-27	
	+	synaptosomes	36.9 \pm 1.1 (10)	45.7 \pm 2.3 (12)	+24
	-		28.1 \pm 0.7 (16)	27.3 \pm 1.5 (11)	NS
		% change	-24	-40	
Frontal cortex	+	slices	85.6 \pm 3.9 (19)	114.6 \pm 6.2 (14)*	+34 ^a
	-		34.7 \pm 2.9 (6)	39.2 \pm 5.1 (7)	NS
		% change	-60	-66	
	+	synaptosomes	27.6 \pm 1.3 (14)	37.5 \pm 1.4 (16)*	+36
	-		18.6 \pm 0.8 (12)	15.9 \pm 0.4 (16)*	-15
		% change	-33	-58	

^aData from Borkowska et al. (1997). Results are mean values \pm SEM. Number of independent experiments in parentheses. Differences HE vs. control: * $P < 0.01$; NS, statistically not significant.

intracellular Ca^{2+} . The present key finding is that irrespective of the brain region (striatum or cerebral cortex), type of preparation (slices or synaptosomes) and mode of superfusion (slices were shaken and continuously oxygenated, whereas synaptosomes were not) HE stimulates only the K^+ -evoked and Ca^{2+} -dependent exocytosis of dopamine. This effect was also independent of the relative magnitude of K^+ -stimulated [^3H]dopamine release in a given preparation. The only HE-evoked change in dopamine release in Ca^{2+} -free medium was a moderate depression of the stimulatory effect of K^+ in frontal cortical synaptosomes. This moderate decrease in Ca^{2+} -independent release confined to one structure and one compartment is unlikely to counterbalance the increased Ca^{2+} -dependent release in the other structures and compartments. The absence of differences between the magnitude of the release in the presence and absence of Ca^{2+} in striatal slices from control rats is intriguing. It is likely to reflect complex intercellular relations in the striatum and/or the complexity of the mechanisms and pools involved in dopamine release from striatal slices (see Vizi 1998, for discussion).

The mechanism by which HE increases the K^+ -evoked, Ca^{2+} -dependent release of dopamine remains to be elucidated. HE in the present model does not affect the uptake of [^3H]dopamine to isolated synaptic vesicles (Albrecht et al. 1994). One molecule which may be considered as a mediator is nitric oxide (NO), which has been found to modulate dopamine synthesis in different CNS preparations (see below). NO synthesis has been found to be increased in a chronic HE model (Rao et al. 1995) and inhibition of NO synthase attenuates symptoms of acute ammonia toxicity (Kosenko et al. 1998). However, the findings on the mode of NO effects on dopamine release are contradictory. On one hand, NO has been found to potentiate the KCl-stimulated release of dopamine in hippocampal slices (Stout and Woodward 1994). On the other, NO has attenuated the KCl-induced Ca^{2+} influx and Ca^{2+} -mediated dopamine release from PC12 cells (Sun et al. 1995). Moreover, NO increases the extracellular accumulation of dopamine by a Ca^{2+} -independent mechanism (Stewart et al. 1996, Kiss et al. 1999), possibly by interacting directly with the dopamine carrier at the plasma membrane (Kiss et al. 1999). Had this mechanism operated in the present HE model, the basal release of [^3H]dopamine should also have been increased, but this was not the case.

Another mediator of the effect of HE on dopamine release to be considered is cAMP. In PC12 cells elevated

K^+ evokes a dopamine release which coincides with an increase in intracellular cAMP (Baizer and Weiner 1985). Acute or chronic treatment with ammonia also increases in a dose-dependent manner the cAMP content in retinal Müller cells in culture (Faff et al. 1996, 1997). The K^+ -evoked, Ca^{2+} -dependent release of dopamine may also be mediated by the released glutamate acting at the NMDA receptors (Giorguieff et al. 1977, Roberts and Anderson 1979, Ochi et al. 1995). The increased release of dopamine in HE may thus be subsequent to the increased release of glutamate. In concert with this hypothesis, the K^+ -evoked accumulation of extracellular glutamate *in vivo* has been seen to increase in the striatum of rats with thioacetamide-induced HE (Hilgier et al. 1999).

Long-term enhancement of dopamine release has been shown to downregulate D_2 receptors in the striata of chronic HE patients (Mousseau et al. 1993) and in dogs with portal-systemic encephalopathy (Zeneroli et al. 1991). This receptor depression is thought to contribute to inhibition of the dopaminergic tone and thus to impaired motor activity in these chronic conditions. Experiments aimed at establishing whether the present relatively short-term treatment with a hepatotoxin affects dopamine receptors are underway in our laboratories.

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