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# Locally infused taurine, GABA and homotaurine alter differently the striatal extracellular concentrations of dopamine and its metabolites in rats

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**Summary.** We studied *in vivo* the effects of locally infused taurine (50, 150, and 450 mM) on the striatal dopamine and its metabolites in comparison with those of GABA and homotaurine, a $GABA_A$ receptor agonist, in freely moving rats. The extracellular dopamine concentration was elevated maximally 2.5-, 2- and 4-fold by taurine, GABA and homotaurine, respectively. At 150 mM concentration, at which the maximum effects occurred, homotaurine increased the extracellular dopamine more than taurine or GABA. When taurine and GABA were infused simultaneously with tetrodotoxin the output of dopamine did not differ from that in the presence of tetrodotoxin alone. In comparison, tetrodotoxin did not inhibit the increase in extracellular dopamine caused by homotaurine. Furthermore, omission of calcium from the perfusion fluid inhibited the increase of extracellular dopamine caused by GABA. However, it did not block the increase of dopamine caused by taurine or homotaurine. The present study suggests that the effects of intrastriatal taurine, GABA and homotaurine on the striatal extracellular dopamine differ. Thus, these amino acids seem to affect the striatal dopaminergic neurons via more than one mechanism.

**Keywords:** Amino acids – Striatal dopamine release – Intrastriatal taurine – GABA – Homotaurine – Microdialysis – Rat

## Introduction

The role of taurine (2-aminoethanesulphonic acid) in the central nervous system is as yet unclear, although it is of interest to note its similarities to the inhibitory neurotransmitters $\gamma$-aminobutyric acid (GABA) and glycine (Simmonds, 1986; Huxtable, 1989). So far a definitive demonstration of the

presence of high affinity sodium-independent binding sites for taurine is lacking (Huxtable, 1989). Taurine and its synthetic enzyme, cysteine sulphonic acid decarboxylase, are found in high concentration in the striatum and in the substantia nigra (Lombardini, 1976; Spears and Martin, 1982; Tossman et al., 1986). At least a portion of striatal taurine is associated with neurons destroyed by kainic acid (Nicklas et al., 1979). Kainic acid lesion also decreased $K^+$-stimulated release of striatal taurine in a microdialysis study (Butcher et al., 1987). Microdialysis as well as *in vitro* studies show that veratridine-stimulated release of taurine is blocked by tetrodotoxin (Butcher and Hamberger, 1987; Della Corte et al., 1990). However, basal release of striatal taurine has not been found to be sensitive to tetrodotoxin (Westerink et al., 1987a; Semba et al., 1995). A high affinity uptake of taurine has been described both in the striatum and in the substantia nigra (Clarke et al., 1983; Della Corte et al., 1987; 1990).

Earlier *post mortem* studies indicate that taurine inhibits the release of striatal dopamine and increases its synthesis in a similar way to GABA and homotaurine, a $GABA_A$ receptor agonist (Garcia de Yebenes Prous et al., 1978; Ahtee and Vahala, 1985; Panula-Lehto et al., 1992). Also, taurine is found to bind to $GABA_A$ as well as to $GABA_B$ receptors (Malminen and Kontro, 1986; Kontro and Oja, 1990; Bureau and Olsen, 1991; Quinn and Harris, 1995). In our previous microdialysis study in anaesthetised rats intranigrally infused taurine decreased extracellular dopamine in the striatum confirming the inhibitory role of taurine in the control of nigrostriatal dopaminergic neurons, whereas local infusion of taurine into the striatum increased extracellular dopamine (Ruotsalainen et al., 1996). Furthermore our recent study revealed that intrastriatal taurine seems to release dopamine by exocytosis since its effect was blocked by simultaneous tetrodotoxin infusion (Ruotsalainen and Ahtee, 1996).

Our main aim was to see whether the effect of local taurine on striatal dopamine resembles those of GABA and homotaurine *in vivo* as it does in *post mortem* studies (Panula-Lehto et al., 1992). Earlier local application of muscimol, a $GABA_A$ agonist, has been shown to increase extracellular DA and its metabolites in the striatum of conscious rats (Yoshida et al., 1993). *In vivo* effects of homotaurine, a structural analogue of taurine and GABA, on extracellular dopamine and its metabolites have not been previously evaluated. The present study was undertaken to clarify the stimulatory effect of local taurine infusion on striatal dopamine and its metabolites using microdialysis in freely moving rats. Further, the action potential dependency of the amino acid-induced release of striatal dopamine was studied by continuously infusing the sodium channel blocker, tetrodotoxin, intrastriatally as well as by omitting calcium from perfusion fluid (Westerink et al., 1987b; 1988). Previously, striatal infusion of hypertonic NaCl solution was found to elevate dopamine levels in striatal dialysates (Horn et al., 1995). Since taurine is involved in osmoregulation in the brain (Wade et al., 1988), control experiments were performed using local hypertonic mannitol infusions.

## Material and methods

### *Animals*

Male Wistar rats weighing 200–350 g (age 9–11 weeks) were housed in groups of 5–6 with free access to tap water and standard rat diet. Rats were maintained in an ambient temperature of 24 ± 2°C and on 12/12 h light/dark cycle (lights on at 6 a.m.). After surgery they were kept individually in their own cages. The experimental set-up was approved by the Provincial Veterinary Officer in the Provincial Government of Uusimaa.

### *Surgery and in vivo sampling procedure*

We used modified I-shaped microdialysis probes (Robinson and Whishaw 1988; Santiago and Westerink 1990). The dialysis membrane we used is the AN69 polyacrylonitrile/sodiummethalyl-sulfonate copolymer (Filtral20, o.d./i.d. 310/220 $\mu$m, Hospal, France). The exposed length of dialysis membrane of the probes was 4 mm. The remaining area of the dialysis tubing was covered by silicone glue to prevent dialysis extraction from regions other than the intended. The rats were anaesthetised with pentobarbitone (pentobarbitone sodium 60–80 mg/kg i.p.). In addition, local lidocaine (5 mg/ml) anaesthesia was used. The rats were then mounted into a Kopf stereotaxic instrument. The skull was exposed and burr holes were drilled. Striatal coordinates were calculated relative to the bregma and dura (A +1.0, L +2.7, D −6.0) according to Paxinos and Watson (1986). If needed, dura was removed with a sharp needle. Two anchor screws were positioned in bone nearby. Before insertation into the brain the dialysis probes were perfused with Ringer solution. The dialysis probe was positioned in the burr hole under stereotaxic guidance. The probe was then slowly lowered down into the brain tissue until it reached its final position. The probe was cemented in this position with dental cement (Aqualox, VOCO, Cuxhaven, Germany). After fixation the probe was again perfused with a small amount of Ringer solution and its inlet and outlet were sealed with plastic caps. The rats were allowed to recover from surgery for 42–46 h.

On the day of the dialysis experiment the rats were connected to a microperfusion pump (Harvard "22", Harvard Apparatus, Mass., U.S.A.). The pump was set to a perfusion speed of 2 $\mu$l/min and 40 $\mu$l samples were collected every 20 min. A 35-$\mu$l aliquot of each sample was injected into the chromatograph. The perfusion medium was a modified Ringer solution containing in mM: NaCl 147, $CaCl_2$ 1.2, KCl 2.7, $MgCl_2$ 1.0 and ascorbic acid 0.04. When calcium was omitted from perfusion fluid the $CaCl_2$ was replaced by NaCl and 1 mM EDTA was added in the perfusion fluid. Drug infusions were started after a stable baseline (four consecutive samples with stable dopamine levels) were obtained. Control rats were infused with Ringer solution throughout the experiment. Effect of hyperosmolarity of infused amino acid solution on the concentrations of extracellular dopamine and its metabolites was tested by infusion of 150 and 450 mM mannitol in Ringer solution.

After the experiments the rats were decapitated. Their brains were rapidly taken out and immersed in phosphate buffer containing 10% formaldehyde. The correct placement of the dialysis probe was verified by eye from sections cut with rodent brain matrix (RBM-4000C, Activational systems Inc., U.S.A.) according to the atlas of Paxinos and Watson (1986). Only results derived from rats with correctly positioned dialysis probes were included in the statistical analysis.

### *Drugs*

The following drugs were dissolved in the perfusion fluid and infused via a microdialysis probe into the striatum: GABA, homotaurine and tetrodotoxin (Sigma Chemical Company, St. Louis, MO, U.S.A.), mannitol (Roquette Freres, Lestren, France), taurine (Fluka AG, Buchs, Switzerland), EDTA (Triplex III, Merck, Darmstadt, Germany).

*Chemical assay*

Dialysates were analyzed immediately after collection. Dopamine, 3,4-hydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were quantified by HPLC with electrochemical detection as earlier described by Ruotsalainen and Ahtee (1996).

*Presentation of results and statistics*

The average of four samples before drug treatment was considered as the baseline level and was defined as 100%. All values given are expressed as percentages of the baseline release. The statistical evaluation of the percentage data was carried out using an analysis of variance (ANOVA) for repeated measurement with one grouping factor (treatment) and one within factor (time). When significant treatment × time interaction (using Huynh-Feldt adjusted degree of freedom) was present, the analysis was continued by performing separate ANOVAS for each pair of treatments between the timepoints of interest using contrasts.

## Results

*Baseline concentrations of striatal extracellular dopamine and its metabolites*

The means (± SEM; n = 142) of the baseline concentrations in the striatal dialysates from rats perfused with the studied drugs and corresponding control rats were as follows: dopamine 221 ± 20 fmol/40 $\mu$l, DOPAC 13.8 ± 0.7 pmol/40 $\mu$l, HVA 9.4 ± 0.5 pmol/40 $\mu$l. No significant differences were found between experiments. The concentrations of dopamine, DOPAC and HVA in dialysates from control rats infused with Ringer solution did not significantly change over the 6-h testing period. It should be noted that the concentrations of amino acids given represent the infused concentrations; the actual content reached in the tissue being smaller. The *in vitro* recoveries of amino acids in the probe used were not determined.

*Effects of hypertonic mannitol infusions*

In a control experiment the effect of elevated osmotic pressure was studied by infusing the rats intrastriatally with 150 an 450 mM mannitol in Ringer solution. The extracellular dopamine tended to decrease at both mannitol concentrations (Table 1). Mannitol at 150 mM decreased extracellular dopamine maximally down to 25% ($P < 0.05$ versus control) and at 450 mM down to 30% ($P < 0.01$ versus control) of the baseline. The extracellular concentrations of DOPAC and HVA were not significantly altered by mannitol infusion.

*Effects of locally infused taurine, GABA and homotaurine*

Local administration of taurine, GABA and homotaurine for 2 h increased the extracellular dopamine in the striatum; dopamine concentrations were maximally 2.5-, 2- and 4-fold the baseline, respectively (Figs. 1,2 and 3). Thus,

**Table 1.** Effects of intrastriatal 150 and 450 mM mannitol infusions on the extracellular concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in rat striatum. Mannitol was added in the perfusion medium at 0 min and continued for 120 min, whereafter the perfusion was continued with control Ringer solution up to 280 min. Changes are expressed as percentages of the baseline release. Statistically significant changes (interactions) in ANOVA for repeated measurement were as follows: DA; control versus mannitol 150 mM $P < 0.05$, mannitol 450 mM $P < 0.01$

| Treatment | n | Baseline (mean ± SEM) | Percentage changes (±SEM) of the baseline release during consecutive 40 min perfusion periods | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 0–40 | 40–80 | 80–120 | 120–160 | 160–200 | 200–240 | 240–280 min |
| DA | | fmol/40 µl | | | | | | | |
| Control | 6 | 232 ± 17 | 91 ± 4 | 91 ± 8 | 92 ± 11 | 96 ± 18 | 88 ± 14 | 92 ± 17 | 81 ± 12 |
| Mannitol 150 | 5 | 282 ± 19 | 74 ± 5 | 59 ± 6 | 51 ± 5 | 35 ± 5 | 29 ± 5 | 28 ± 4 | 25 ± 4 |
| Mannitol 450 | 6 | 238 ± 20 | 78 ± 4 | 80 ± 8 | 51 ± 8 | 30 ± 6 | 38 ± 9 | 45 ± 11 | 57 ± 12 |
| DOPAC | | pmol/40 µl | | | | | | | |
| Control | 6 | 13.7 ± 0.7 | 101 ± 4 | 100 ± 3 | 101 ± 4 | 100 ± 4 | 97 ± 3 | 97 ± 4 | 94 ± 5 |
| Mannitol 150 | 5 | 11.7 ± 0.5 | 111 ± 3 | 112 ± 5 | 116 ± 5 | 118 ± 4 | 113 ± 5 | 116 ± 5 | 113 ± 4 |
| Mannitol 450 | 6 | 12.6 ± 0.7 | 107 ± 2 | 111 ± 4 | 104 ± 6 | 106 ± 7 | 112 ± 9 | 109 ± 6 | 110 ± 6 |
| HVA | | pmol/40 µl | | | | | | | |
| Control | 6 | 8.8 ± 0.4 | 106 ± 7 | 104 ± 5 | 109 ± 5 | 106 ± 6 | 103 ± 3 | 101 ± 4 | 99 ± 4 |
| Mannitol 150 | 5 | 7.4 ± 0.3 | 105 ± 2 | 109 ± 3 | 107 ± 3 | 105 ± 1 | 99 ± 3 | 99 ± 6 | 98 ± 4 |
| Mannitol 450 | 6 | 8.9 ± 0.5 | 106 ± 3 | 121 ± 8 | 107 ± 9 | 101 ± 9 | 99 ± 9 | 88 ± 6 | 94 ± 7 |

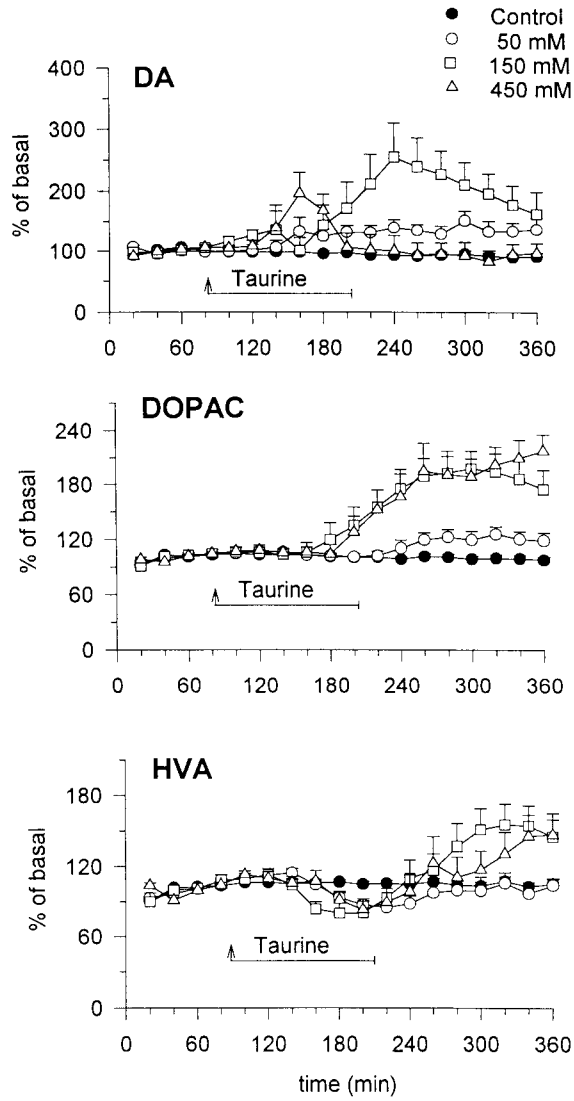


**Fig. 1.** Effects of intrastriatal taurine (50, 150 and 450mM for 2h, 2$\mu$l/min) on the extracellular concentrations of dopamine and its metabolites (DOPAC and HVA) in the striatum of conscious rats. The data are given as means ± SEM (n = 5–20), and expressed as percentages of the baseline. Statistically significant changes (interaction) in ANOVA for repeated measures were following: *DA* (dopamine), control versus taurine 150mM $P < 0.001$, 450mM $P < 0.01$; taurine 50mM versus 150mM $P < 0.01$, 450mM $P < 0.05$; taurine 150mM versus 450mM $P < 0.001$. *DOPAC*, control versus taurine 150mM $P < 0.001$, 450mM $P < 0.001$; taurine 50mM versus 150mM $P < 0.001$, 450mM $P < 0.001$; taurine 150mM versus 450mM $P < 0.05$. *HVA*, control versus taurine 150mM $P < 0.001$, 450mM $P < 0.001$; taurine 50mM versus 150mM $P < 0.001$, 450mM $P < 0.01$; taurine 150mM versus 450mM $P < 0.01$

at 150mM concentration, at which the maximum effects occurred, homotaurine elevated the extracellular dopamine significantly more than taurine ($P < 0.001$) or GABA ($P < 0.01$). At the 450mM concentration taurine and homotaurine elevated extracellular dopamine clearly less than at

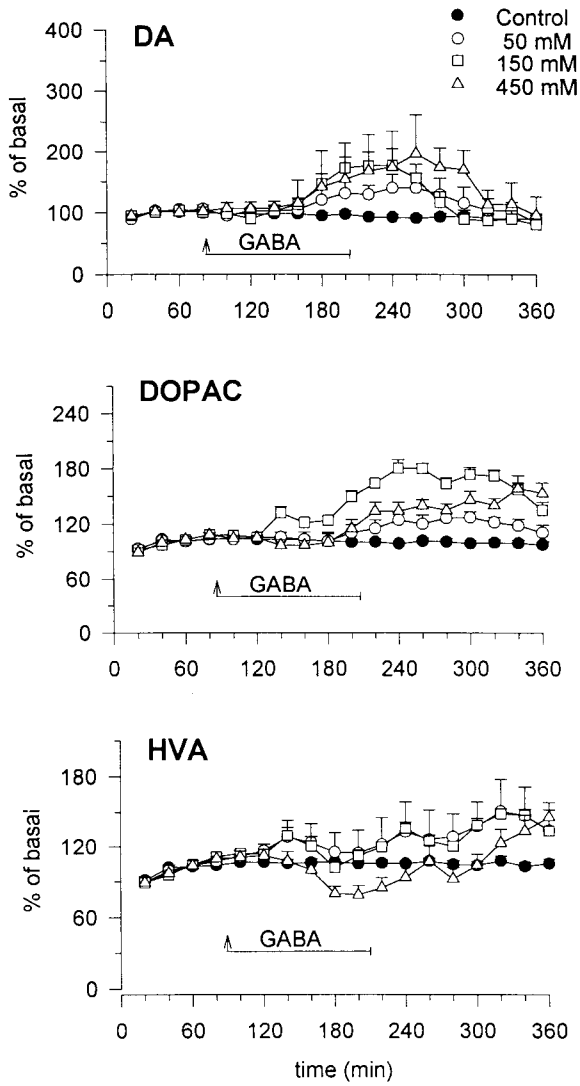


**Fig. 2.** Effects of intrastriatal GABA (50, 150 and 450mM for 2h, 2$\mu$l/min) on the extracellular concentrations of dopamine and its metabolites (DOPAC and HVA) in the striatum of conscious rats. The data are given as means ± SEM (n = 5–20), and expressed as percentages of the baseline. Statistically significant changes (interaction) in ANOVA for repeated measures were following: *DA* (dopamine), control versus GABA 150mM $P < 0.001$, 450mM $P < 0.001$. *DOPAC*, control versus GABA 50mM $P < 0.001$, 150mM $P < 0.001$, 450mM $P < 0.001$; GABA 50mM versus 150mM $P < 0.001$, 450mM $P < 0.001$; GABA 150mM versus 450mM $P < 0.001$. *HVA*, control versus GABA 50mM $P < 0.001$, 150mM $P < 0.001$,450mM $P < 0.001$; GABA 50mM versus 450mM $P < 0.001$; GABA 150mM versus 450mM $P < 0.001$

150mM, but the effect of GABA was about similar at both concentrations. Furthermore, the increase caused by the highest taurine concentration was short lasting. Dopamine release was not significantly increased by the 50mM concentration of any of the amino acids. At the concentrations used the amino acids did not cause any notable alterations in the behaviour of the animals.

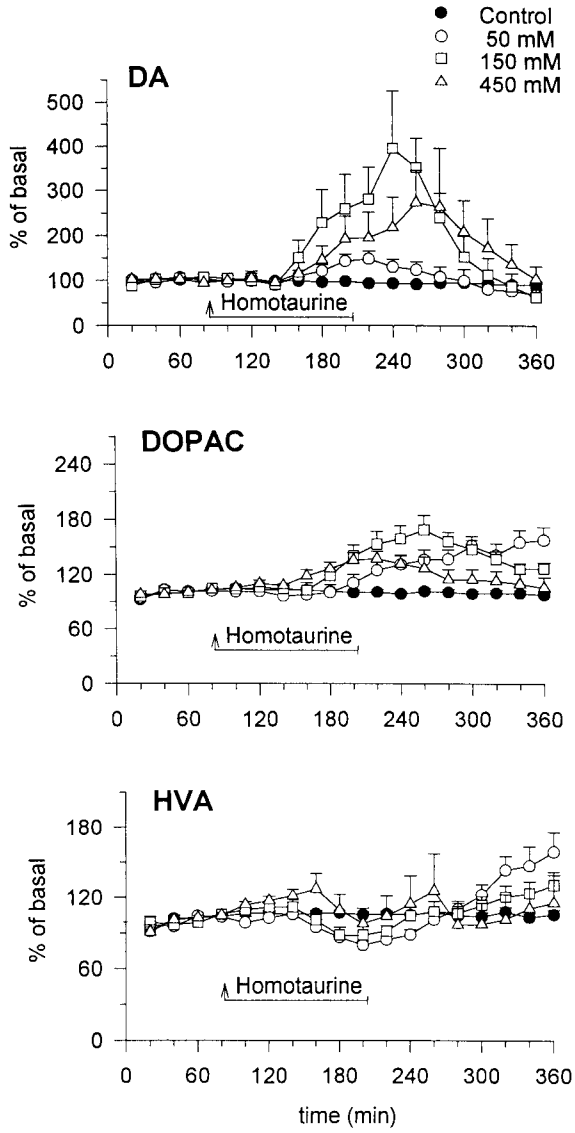


**Fig. 3.** Effects of intrastriatal homotaurine (50, 150 and 450 mM for 2 h, 2 $\mu$l/min) on the extracellular concentrations of dopamine and its metabolites (DOPAC and HVA) in the striatum of conscious rats. The data are given as means ± SEM (n = 5–20), and expressed as percentages of the baseline. Statistically significant changes (interaction) in ANOVA for repeated measures were following: *DA* (dopamine), control versus homotaurine 150 mM $P < 0.001$, 450 mM $P < 0.05$; homotaurine 50 mM versus 150 mM $P < 0.01$, homotaurine 150 mM versus 450 mM $P < 0.05$. *DOPAC*, control versus homotaurine 50 mM $P < 0.001$, 150 mM $P < 0.001$, 450 mM $P < 0.001$; homotaurine 50 mM versus 150 mM $P < 0.001$, 450 mM $P < 0.001$; homotaurine 150 mM versus 450 mM $P < 0.001$. *HVA*, control versus homotaurine 50 mM $P < 0.001$, 150 mM $P < 0.01$; homotaurine 50 mM versus 150 mM $P < 0.05$, 450 mM $P < 0.001$; homotaurine 150 mM versus 450 mM $P < 0.01$

Extracellular DOPAC increased maximally up to 200%, 180% and 170% by 150mM concentration of taurine, GABA and homotaurine, respectively (Figs. 1,2 and 3). After 450mM taurine the DOPAC increase lasted slightly longer than after the 150mM concentration, but the DOPAC increases caused by the 450mM GABA or homotaurine were smaller than those by 150mM. GABA and homotaurine increased DOPAC also at 50mM although less than at the higher concentrations. The 50mM taurine did not affect extracellular DOPAC.

All amino acids affected extracellular HVA (Figs. 1,2 and 3). During the infusion of the amino acids extracellular HVA initially tended to decrease. This was most clearly seen during taurine and homotaurine infusions; GABA decreased HVA only at the 450mM concentration. After the infusion of 150 and 450mM taurine HVA increased maximally up to 155%. GABA elevated extracellular HVA maximally up to 150%. At the 50 and 150mM concentrations homotaurine increased extracellular HVA maximally to 160% after infusion; 450mM homotaurine did not significantly alter extracellular HVA.

### *Effects of tetrodotoxin on taurine-, GABA- and homotaurine-induced changes*

Intrastriatal tetrodotoxin (1$\mu$M) infusion decreased extracelluar dopamine concentration to 10% of the control, but did not significantly alter DOPAC or HVA (Fig. 4A). The infusion of taurine and GABA (both at 150mM concentration) 60 minutes after the start of tetrodotoxin infusion (Fig. 4B and C) produced no significant effect. On the other hand, the infusion of 150mM homotaurine resulted in an increase in extracellular dopamine (Fig. 4D), similar to the effects of homotaurine in the absence of tetrodotoxin (Fig. 3). Tetrodotoxin abolished the effects of taurine on dopamine metabolites as well as that of homotaurine on DOPAC (Fig. 4B and D). Moreover, during tetrodotoxin infusion homotaurine did not increase extracellular HVA (Fig. 4D). Tetrodotoxin blocked only partially the effects of GABA on DOPAC and HVA (Fig. 4C).

### *Effects of omission of calcium on taurine-, GABA- and homotaurine induced changes*

Omission of calcium from and adding EDTA (1mM) in the perfusion fluid decreased extracellular dopamine concentration to about 10% of the control (Fig. 5A). Metabolites DOPAC and HVA were also decreased by 25% and 35%, respectively. Infusion of taurine (at 150mM concentration) after omission of calcium resulted in increases in extracellular dopamine, DOPAC and HVA (Fig. 5B), similar to the effects seen in Fig. 1. In contrast, infusion of 150mM GABA after omission of calcium produced no effect on dopamine levels and only a slight reversal of the effects of calcium omission on DOPAC and HVA levels (Fig. 5C). In a similar way to tetrodotoxin, omission of calcium did not affect the 150mM homotaurine-induced elevation of

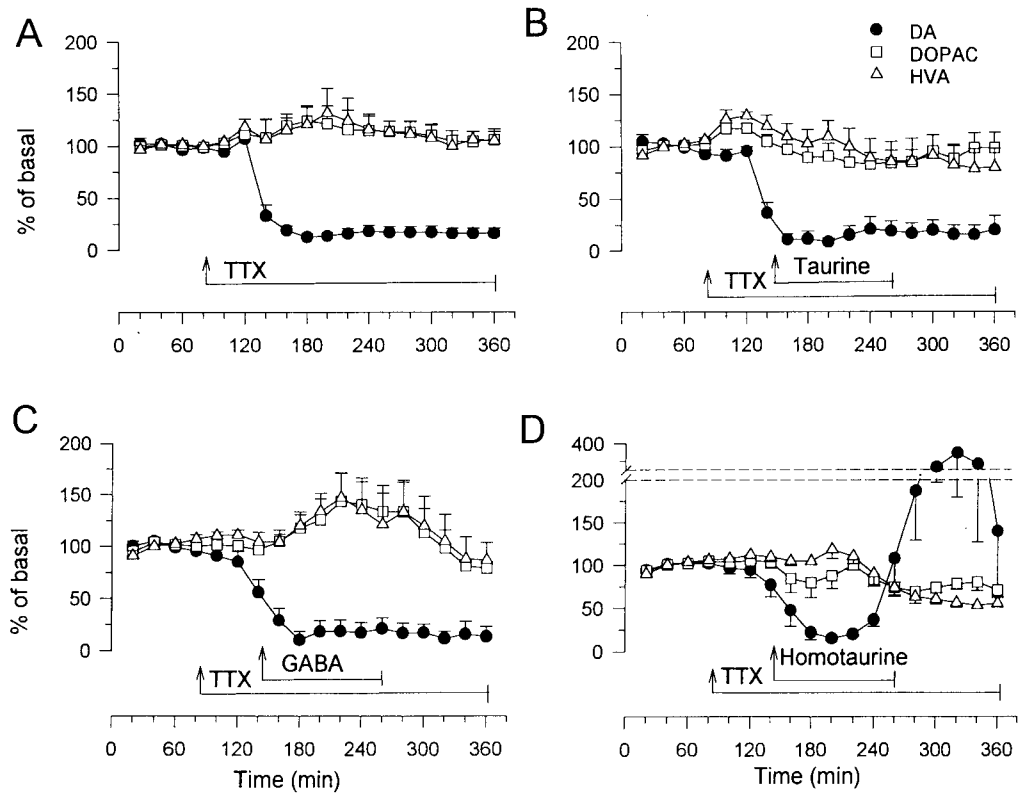


**Fig. 4.** Effect of intrastriatal taurine (**B**), GABA (**C**) and homotaurine (**D**) (150 mM for 2 h, 2 $\mu$l/min) on the extracellular concentrations of dopamine, DOPAC and HVA in striatal dialysates during simultaneous tetrodotoxin (**A**, TTX, 1 $\mu$M) infusion. Intrastriatal TTX infusion was started 60 min before amino acids were added into the perfusion fluid and continued until the end of experiment. The data are given as means ± SEM (n = 5). The double dotted line in Fig. **D** indicates a change in the scale of the ordinate. The effects of intrastriatal Ringer infusion, taurine 150 mM, GABA 150 mM and homotaurine 150 mM are shown in Figs. 1–3. Statistically significant changes (interaction) as compared with corresponding controls in ANOVA for repeated measures were following: *Dopamine*, control (intrastriatal Ringer infusion; not shown) versus TTX (**A**) $P < 0.001$; taurine (150 mM) versus TTX + taurine (**B**) $P < 0.05$; GABA (150 mM) versus TTX + GABA (**C**) $P < 0.001$. *DOPAC*, taurine (150 mM) versus TTX + taurine (**B**) $P < 0.001$; GABA (150 mM) versus TTX + GABA (**C**) $P < 0.001$; homotaurine (150 mM) versus TTX + homotaurine (**D**) $P < 0.001$. *HVA*, taurine (150 mM) versus TTX + taurine (**B**) $P < 0.001$; GABA (150 mM) versus TTX + GABA (**C**) $P < 0.01$; homotaurine (150 mM) versus TTX + homotaurine (**D**) $P < 0.001$

extracellular dopamine, but it slightly altered the time-courses of homotaurine-induced changes in extracellular DOPAC and HVA as shown statistically significant changes (Fig. 5D).

## Discussion

In the present study we have shown that intrastriatal taurine as well as GABA and homotaurine elevate striatal extracellular dopamine concentration *in*

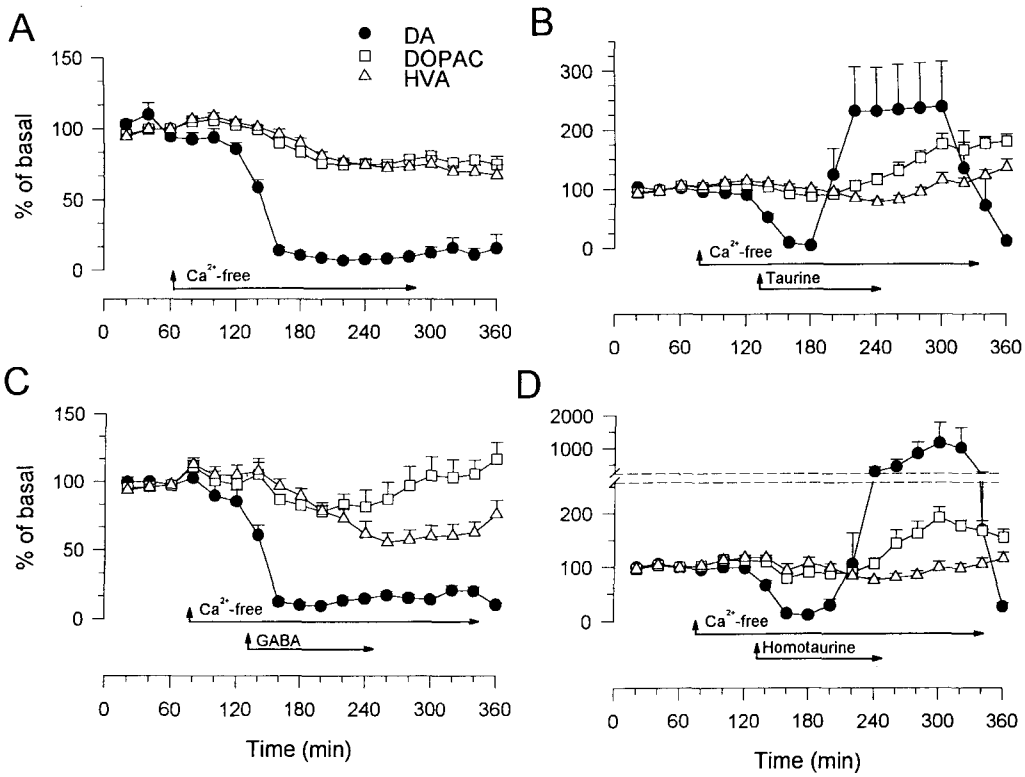


**Fig. 5.** Effect of intrastriatal taurine (**B**), GABA (**C**) and homotaurine (**D**) (150 mM for 2 h, 2 $\mu$l/min) on the extracellular concentrations of dopamine, DOPAC and HVA in striatal dialysates during removal of calcium and presence of 1 mM EDTA in perfusion fluid (Ca-free) (**A**). Calcium was omitted from the perfusion fluid 60 min before amino acids were added into the perfusion fluid. The data are given as means ± SEM (n = 5–6). For another explanations see legend for Fig. 4. Statistically significant changes (interaction) as compared with corresponding controls in ANOVA for repeated measures were following: *Dopamine*, control (intrastriatal Ringer infusion; not shown) versus Ca-free (**A**) $P < 0.001$; GABA (150 mM) versus Ca-free + GABA (**C**) $P < 0.01$. *DOPAC*, control (intrastriatal Ringer infusion) versus Ca-free (**A**) $P < 0.001$, GABA (150 mM) versus Ca-free + GABA (**C**) $P < 0.05$; homotaurine (150 mM) versus Ca-free + homotaurine (**D**) $P < 0.05$. *HVA*, control (intrastriatal Ringer infusion) versus Ca-free (**A**) $P < 0.001$, GABA (150 mM) versus Ca-free + GABA (**C**) $P < 0.06$; homotaurine (150 mM) versus Ca-free + homotaurine (**D**) $P < 0.05$

*vivo*. Of the three amino acids homotaurine increased the extracellular dopamine most and GABA least. Furthermore, the amino acids studied increased the dopamine metabolites, DOPAC and HVA in the striatal extracellular fluid. Tetrodotoxin-infusion during brain dialysis and omission of calcium together with inclusion of EDTA into the perfusion fluid are used to study whether drug-induced dopamine release is caused by exocytosis (Westerink et al., 1987b; 1988). In the present study tetrodotoxin-sensitivities of the effects of GABA and taurine imply that these amino acids increase dopamine release in the striatum impulse-flow dependently, whereas homotaurine-induced increase in extracellular dopamine does not seem to be

dependent on the opening of voltage-dependent sodium-channels. Omission of calcium confirmed the exocytotic nature of GABA-induced dopamine release, while taurine- and homotaurine-induced elevations of extracellular dopamine were not sensitive to omission of calcium.

Though our present findings that intrastriatal GABA increased extracellular dopamine seem to be contradictory to the inhibitory role of GABA, other studies show similar results. The stimulatory effect of GABA and $GABA_A$ receptor agonist, muscimol, on striatal dopamine release has previously been demonstrated in rat striatal slice preparations (Giorguieff et al., 1978; Kerwin and Pycock, 1979; Stoof et al., 1979). Also, local GABA stimulated striatal ($^3$H)-dopamine release *in vivo* in push-pull cannula experiments in cats (Chéramy et al., 1978). Further, stimulation of dopamine release was markedly reduced in the presence of picrotoxin implying that the effect is mediated by $GABA_A$ receptors (Chéramy et al., 1978; Giorguieff et al., 1978). Chéramy et al. (1978) suggested that this effect of GABA could have been mediated by GABA receptors located on interneurons or neuronal afferent fibers in contact with the dopamine terminals since it was no longer observed in the presence of tetrodotoxin. Also in *in vivo* experiments kainate lesions of the striatum inhibited the stimulatory effects of intrastriatal muscimol on dopamine release supporting the suggestions that a population of interneurons might be involved (Wood, 1982; Yoshida et al., 1993). Confusingly, locally administered $GABA_A$ antagonist, bicuculline, also elevated striatal extracellular dopamine. Bicuculline failed to increase striatal dopamine after kainic acid lesion confirming that it acts on receptors which seem to be postsynaptic and located on intrastriatal interneurons or neurons of the striatonigral feedback loop (Smolders et al., 1995). In our experiments both tetrodotoxin and omission of calcium were able to block GABA-induced dopamine release *in vivo* confirming that GABA releases dopamine impulse-flow dependently. Previously Ennis and Cox (1981) have shown that tetrodotoxin is able to prevent GABA-induced enhancement of $K^+$-evoked release of ($^3$H)-dopamine from preloaded striatal slices.

In the present study GABA-induced increase of extracellular dopamine was not further enhanced by increasing the amino acid concentration from 150 mM to 450 mM, and taurine and homotaurine increased extracellular dopamine even less at 450 mM than at 150 mM concentration. The ceiling effect of GABA-induced elevation of extracellular dopamine might be due to its actions on different GABA-sensitive receptors in the striatum. As discussed above muscimol has been found to increase dopamine release, whereas activation of $GABA_B$ receptors by intrastriatal baclofen decreases extracellular dopamine (Smolders et al., 1995). GABA was least effective of the three amino acids studied to increase extracellular dopamine. The reason for smaller effects by GABA might be related to the high efficacy of its removal from extracellular space after local administration. Thus, the high affinity uptake of GABA is more effective than that of taurine (Debler and Lajtha, 1987). Furthermore, taurine is not metabolized in the brain (Huxtable, 1989). This might be as well the explanation for higher efficacy of homo-

taurine, which as a synthetic compound is probably neither metabolized in the brain nor effectively taken up by neurons or glial cells.

Homotaurine has been shown to be a potent $GABA_A$ receptor agonist (Bowery et al., 1979). However, homotaurine did not increase $^3$H-diazepam binding like GABA indicating that homotaurine might not be a full agonist at all $GABA_A$ receptor subtypes (Braestrup et al., 1979; Falch et al., 1985). In the present experiment intrastriatal homotaurine caused the largest elevation of striatal extracellular dopamine of the three amino acids studied. This agrees with our earlier studies (Ahtee and Vahala, 1985; Panula-Lehto et al., 1992) showing that homotaurine is more effective than taurine or GABA in increasing the striatal dopamine metabolism after intracerebral administration. In contrast with the finding showing that tetrodotoxin abolished the homotaurine stimulated release of newly-synthetized ($^3$H)dopamine in striatal slices (Giorguieff-Chesselet et al., 1979), in the present experiment homotaurine-induced increase of extracellular dopamine was blocked neither by tetrodotoxin nor by omission of calcium. These findings imply that the mechanism by which homotaurine increases concentration of extracellular dopamine is neither impulse-flow dependent nor by exocytosis and, furthermore, that it is not similar to that of GABA. One possibility is that homotaurine, in addition to its effects on GABAergic receptor, interacts with dopamine uptake system. The finding that at 450 mM homotaurine increased extracellular dopamine significantly less than at 150 mM suggests that at large doses other effects of homotaurine might be predominant.

Taurine increased striatal extracellular dopamine as earlier shown in anaesthetised as well as in freely moving rats (Ruotsalainen et al., 1996; Ruotsalainen and Ahtee, 1996). Furthermore, taurine-induced release of striatal dopamine is shown to be sensitive to tetrodotoxin (Ruotsalainen and Ahtee, 1996). In contrast to this the present study revealed that omission of calcium was not able to block taurine-induced dopamine release. Thus calcium does not seem to be essential to action of taurine on dopamine release. One possibility is that omission of calcium from and adding EDTA into perfusion fluid is not able to remove calcium effectively. Since calcium is freely diffusible it obviously diffuses from surrounding areas towards the dialyzed brain area. Others have previously concluded that for estimation of nerve impulse dependent release use of calcium-free perfusion fluid is not as sensitive as the use of tetrodotoxin (Santiago and Westerink, 1990). However, our present finding that omission of calcium was as effective as tetrodotoxin to reduce basal dopamine release as well as to inhibit the effect of GABA does not agree with this explanation. Another explanation for these contradictory findings is that taurine as a regulator of $Ca^{2+}$ homeostasis (Huxtable, 1989) counteracts the effects of the reduction of extracellular $Ca^{2+}$ by elevating intracellular $Ca^{2+}$.

Binding studies indicate that taurine might act on both $GABA_A$ and glycine receptors (Pasantes-Morales, 1981; Simmonds, 1986; Huxtable, 1989; Bureau and Olsen, 1991; Häusser et al., 1992; Quinn and Harris, 1995). This is also supported by *in vitro* studies using Xenopus oocytes injected with mouse brain messenger RNA of glycine and GABA receptors (Horikoshi et al.,

1988; Wahl et al., 1994). Similarly to GABA glycine stimulated striatal dopamine release after intrastriatal administration in conscious rats (Yadid et al., 1993). However, at the present it is not clear what is taurine's mechanism of action. Its effects on striatal dopaminergic neurons might also involve mechanisms not related to receptor activation particularly at higher doses. Increasing the taurine concentration did not enhance dopamine release; instead at the highest 450 mM concentration taurine caused only shortlasting increase of dopamine. Similar, although bigger, instantaneous increase of dopamine was seen in anaesthetised rats using 450 mM concentration (Ruotsalainen et al., 1996).

Intrastriatal taurine, GABA and homotaurine infusions elevated the extracellular dopamine metabolite DOPAC. The maximum elevations of DOPAC by GABA and homotaurine occurred at 150 mM concentrations, and at 450 mM these amino acids elevated DOPAC significantly less. In contrast, 150 and 450 mM taurine infusions caused a similar elevation of DOPAC, although 450 mM taurine caused only short lasting increase in dopamine release. Furthermore, the amino acids tended to increase DOPAC also at 50 mM concentration although dopamine was not significantly affected. We have previously found taurine, GABA and homotaurine to increase DOPAC levels after i.c.v. administration (Panula-Lehto et al., 1992). Present findings agree also with previous reports that taurine in anaesthetised rats (Ruotsalainen et al., 1996) and muscimol in conscious rats (Yoshida et al., 1993) elevate extracellular DOPAC after intrastriatal infusion. The dopamine metabolite, HVA, was initially decreased, but was increased by all amino acids after the end of their infusion. We have previously found that intrastriatal taurine decreases HVA in anaesthetised rats (Ruotsalainen et al., 1996). Major portion of extracellular DOPAC is derived from an intraneuronal pool of newly synthesized dopamine and HVA might reflect changes in DOPAC (Westerink, 1985; Zetterström et al., 1988); increase in HVA after amino acid infusions could be due to increase in DOPAC, and thus, an increase in intraneuronal metabolism of dopamine. The metabolite levels may be functionally dissociated from neuronal activity or neurotransmitter release. Furthermore, many factors determine the steady state interstitial dopamine metabolite levels. Therefore, increased HVA levels in dialysates might as well be due to blockade of conjugation and/or the acid carrier, rather than simply reflecting its increased formation from dopamine (Commissiong, 1985; Cumming et al., 1992).

As we have previously found (Ruotsalainen and Ahtee, 1996) tetrodotoxin blocked taurine-induced changes in dopamine metabolites. Omission of calcium, which in contrast to tetrodotoxin did not affect taurine-induced increase in dopamine, had no effect on taurine-induced changes in DOPAC and HVA. GABA-induced changes in dopamine metabolites seem to be only partially related to the increased dopamine release since tetrodotoxin and omission of calcium were able to block dopamine release but only partially blocked the increases in extracellular metabolites. Tetrodotoxin infusion abolished homotaurine-induced changes in dopamine metabolites, although this treatment did not affect the homotaurine induced elevation of

extracellular dopamine. However, omission of calcium was not able to reverse the effects of homotaurine on DOPAC and HVA as clearly as tetrodotoxin did. These findings imply that homotaurine-induced changes in extracellular DOPAC and HVA are partially related to processes connected with impulse-flow dependent dopamine release.

Control experiments, using 150 and 450 mM mannitol to increase osmotic pressure of the infused Ringer solution instead of amino acids showed that elevating osmotic pressure reduces the release of dopamine without affecting the extracellular concentrations of its metabolites. Thus these findings confirmed that the observed elevation of extracellular dopamine or changes in dopamine metabolites are not due to hyperosmolarity of the infused solutions. However, it cannot be excluded that the smaller elevation of extracellular dopamine by taurine and homotaurine at 450 mM than at 150 mM concentration is related to the elevation of osmotic pressure. Perfusion with high-NaCl solution (1 M) has been shown to elevate striatal extracellular dopamine (Horn et al., 1995). Effect of high-NaCl results probably from a direct effect of the $Na^+$-ion on dopaminergic terminals or from an interaction of amino acids with dopaminergic neurons. High-NaCl also increased the release of striatal amino acids while hyperosmolar mannitol decreased predominantly the extracellular concentration of excitatory amino acids in the striatum (Horn et al., 1995). At present we cannot explain the decrease of dopamine after hyperosmotic mannitol infusion observed in the present study, but it could be related to the reduction of extracellular excitatory amino acids.

In conclusion, our experiments show that locally infused taurine, GABA and homotaurine elevate striatal extracellular dopamine *in vivo*. The sensitivity of the effect of GABA to tetrodotoxin and omission of calcium shows that it increases the dopamine release in the striatum impulse-flow dependently, whereas homotaurine seems to increase extracellular dopamine via a tetrodotoxin- and calcium-insensitive mechanism. Taurine-induced elevation of extracellular dopamine was sensitive to tetrodotoxin but not to omission of calcium. Thus its effects are not clearly resembling those of either GABA or homotaurine. However, it might partially share a same mechanism of action with them. Taken together the present findings suggest that taurine, GABA and homotaurine act on nigrostriatal dopaminergic neurons via more than one mechanism.

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## References

Ahtee L, Vahala M-L (1985) Taurine and its derivatives alter brain dopamine metabolism similarly to GABA in mice and rats. In: Oja S, Ahtee L, Kontro P, Paasonen MK (eds)

Taurine: biological actions and clinical perspectives. Alan R. Liss, New York, pp 331–341

Bowery NG, Hill DR, Hudson AL (1983) Characteristics of $GABA_B$ receptor binding sites on rat whole brain synaptic membranes. Br J Pharmacol 78: 191–206

Braestrup C, Nielsen M, Krogsgaard-Larsen P, Falch E (1979) Partial agonists for brain GABA/benzodiazepine receptor complex. Nature 280: 331–333

Bureau MH, Olsen RW (1991) Taurine acts on a subclass of $GABA_A$ receptors in mammalian brain *in vitro*. Eur J Pharmacol 207: 9–16

Butcher SP, Hamberger A (1987) *In vivo* studies on the extracellular, and veratrine-releasable, pools of endogenous amino acids in the rat striatum: effects of corticostriatal deafferentation and kainic acid lesion. J Neurochem 48: 713–721

Butcher SP, Lazarewicz JW, Hamberger A (1987) *In vivo* microdialysis studies on the effects of decortication and excitotoxic lesions on kainic acid-induced calcium fluxes, and endogenous amino acid release, in the rat striatum. J Neurochem 49: 1355–1360

Chéramy A, Nieoullon A, Glowinski J (1978) Gabaergic processes involved in the control of dopamine release from nigrostriatal dopaminergic neurons in the cat. Eur J Pharmacol 48: 281–295

Clarke DJ, Smith AD, Bolam JP (1983) Uptake of [$^3$H]-taurine into medium-size neurons and into identified striatonigral neurons in the rat neostriatum. Brain Res 289: 342–348

Commissiong JW (1985) Monoamine metabolites: their relationship and lack of relationship to monoaminergic neuronal activity. Biochemical Pharmacol 34: 1127–1131

Cumming P, Brown E, Damsma G, Fibiger H (1992) Formation and clearance of interstitial metabolites of dopamine and serotonin in the rat striatum: an *in vivo* microdialysis study. J Neurochem 59: 1905–1914

Debler EA, Lajtha A (1987) High-affinity transport of $\gamma$-aminobutyric acid, glycine, taurine, L-aspartic acid, and L-glutamic acid in synaptosomal ($P_2$) tissue: a kinetic and substrate specificity analysis. J Neurochem 48: 1851–1856

Della Corte L, Clarke DJ, Bolam JP, Smith AD (1987) Uptake, localization and release of taurine in the rat basal ganglia. In: Huxtable RJ, Franconi F, Giotti A (eds) The biology of taurine. Plenum Publ. Corp., New York, pp 285–294

Della Corte L, Bolam JP, Clarke DJ, Parry DM, Smith AD (1990) Sites of [$^3$H]taurine uptake in the rat substantia nigra in relation to the release of taurine from the striatonigral pathway. Eur J Neurosci 2: 50–61

Ennis C, Cox B (1981) GABA enhancement of [$^3$H]dopamine release from slices of rat striatum: dependence on slice size. Eur J Pharmacol 70: 417–420

Falch E, Jacobsen P, Krogsgaard-Larsen P, Curtis DR (1985) GABA-mimetic activity and effects on diazepam binding of aminosulphonic acids structurally related to piperidine-4-sulphonic acid. J Neurochem 44: 68–75

Garcia de Yebenes Prous J, Carlsson A, Mena Gomez MA (1978) The effect of taurine on motor behaviour, body temperature and monoamine metabolism in the rat brain. Naunyn-Schmiedeberg's Arch Pharmacol 304: 95–99

Giorguieff MF, Kemel ML, Glowinski J, Besson MJ (1978) Stimulation of dopamine release by GABA in rat striatal slices. Brain Res 139: 115–130

Giorguieff-Chesselet MF, Kemel ML, Wandscheer D, Glowinski J (1979) Attemps to localize the GABA receptor involved in the GABA-induced release of newly-synthesized [$^3$H]dopamine in rat striatal slices. Brain Res 175: 383–386

Horikoshi T, Asanuma A, Yanagisawa K, Anzai K, Goto S (1988) Taurine and $\beta$-alanine act on both GABA and glycine receptors in Xenopus oocyte injected with mouse brain messenger RNA. Molecular Brain Res 4: 97–105

Horn T, Bauce L, Landgraf R, Pittman QJ (1995) Microdialysis with high NaCl causes central release of amino acids and dopamine. J Neurochem 64: 1632–1644

Huxtable RJ (1989) Taurine in the central nervous system and the mammalian actions of taurine. Prog Neurobiol 32: 471–533

Häusser MA, Yung WH, Lacey MG (1992) Taurine and glycine activate the same $Cl^-$ conductance in substantia nigra dopamine neurones. Brain Res 571: 103–108

Kerwin R, Pycock C (1979) Effects of $\omega$-amino acids on tritiated dopamine release from rat striatum: evidence for a possible glycinergic mechanism. Biochem Pharmacol 28: 2193–2197

Kontro P, Oja SS (1990) Interactions of taurine with $GABA_B$ binding sites in mouse brain. Neuropharmacol 29: 243–247

Lombardini JB (1976) Regional and subcellular studies on taurine in the rat central nervous system. In: Huxtable RJ, Barbeau A (eds) Taurine. Raven Press, New York, pp 311–326

Malminen O, Kontro P (1986) Modulation of the GABA-benzodiazepine receptor complex by taurine in rat brain membranes. Neurochem Res 11: 85–94

Nicklas WJ, Duvoisin RC, Berl S (1979) Amino acids in rat neostriatum: alteration by kainic acid lesion. Brain Res 167: 107–117

Panula-Lehto E, Mäkinen M, Ahtee L (1992) Effects of taurine, homotaurine and GABA on hypothalamic and striatal dopamine metabolism. Naunyn-Schmiedeberg's Arch Pharmacol 346: 57–62

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. Academic Press, New York

Quinn MR, Harris CL (1995) Taurine allosterically inhibits binding of [$^{35}$S]-t-butylbicyclophosphorothionate (TBPS) to rat brain synaptic membranes. Neuropharmacol 34: 1607–1613

Robinson TE, Whishaw IQ (1988) Normalization of extracellular dopamine in striatum following recovery from a partial unilateral 6-OHDA lesion of the substantia nigra: a microdialysis study in freely moving rats. Brain Res 450: 209–224

Ruotsalainen M, Ahtee L (1996) Intrastriatal taurine increases striatal extracellular dopamine in a tetrodotoxin-sensitive manner in rats. Neurosci Lett 212: 175–178

Ruotsalainen M, Heikkilä M, Lillsunde P, Seppälä T, Ahtee L (1996) Taurine infused intrastriatally elevates, but intranigrally decreases striatal extracellular dopamine concentration in anaesthetised rats. J Neural Transm 103: 935–946

Santiago M, Westerink BHC (1990) Characterization of the *in vivo* release of dopamine as recorded by different types of intracerebral microdialysis probes. Naunyn-Schmiedeberg's Arch Pharmacol 342: 407–414

Semba J, Kito S, Toru M (1995) Characterisation of extracellular amino acids in striatum of freely moving rats by *in vivo* microdialysis. J Neural Transm 100: 39–52

Simmonds MA (1986) Classification of inhibitory amino acid receptors in the mammalian nervous system. Med Biol 64: 301–311

Smolders I, De Klippel N, Sarre S, Ebinger G, Michotte Y (1995) Tonic GABA-ergic modulation of striatal dopamine release studied by *in vivo* microdialysis in the freely moving rats. Eur J Pharmacol 284: 83–91

Spears RM, Martin DL (1982) Resolution and brain regional distribution of cysteine sulfinate decarboxylase isoenzymes from hog brain. J Neurochem 38: 985–991

Stoof JC, Den Breejen EJS, Mulder AH (1979) GABA modulates the release of dopamine and acetylcholine from rat caudate nucleus slices. Eur J Pharmacol 57: 35–42

Tossman U, Jonsson G, Ungerstedt U (1986) Regional distribution and extracellular levels of amino acids in rat central nervous system. Acta Physiol Scand 127: 533–545

Wade JV, Olson JP, Samson FE, Nelson SR, Pazdernik TL (1988) A possible role for taurine in osmoregulation within the brain. J Neurochem 51: 740–745

Wahl P, Elster L, Schousboe A (1994) Identification and function of glycine receptors in cultured cerebellar granule cells. J Neurochem 62: 2457–2463

Westerink BHC (1985) Sequence and significance of dopamine metabolism in the rat brain. Neurochem Int 7: 221–227

Westerink BHC, Damsma G, Rollema H, De Vries JB, Horn AS (1987a) Scope and limitations of *in vivo* brain dialysis: a comparison of its application to various neurotransmitter systems. Life Sci 41: 1763–1776

Westerink BHC, Tuntler J, Damsma G, Rollema H, de Vries JB (1987b) The use of tetrodotoxin for the characterization of drug-enhanced dopamine release in conscious rats studied by brain dialysis. Naunyn-Schmiedeberg's Arch Pharmacol 336: 502–507

Westerink BHC, Hofsteede HM, Damsma G, De Vries JB (1988) The significance of extracellular calcium for the release of dopamine, acetylcholine and amino acids in concious rats. Naunyn-Schmiedeberg's Arch Pharmacol 337: 373–378

Wood PL (1982) Actions of GABAergic agents on dopamine metabolism in the nigrostriatal pathway of the rat. J Pharmacol Exp Ther 222: 674–679

Yadid G, Pacak K, Golomb E, Harvey-White JD, Lieberman DM, Kopin IJ, Goldstein DS (1993) Glycine stimulates striatal dopamine release in conscious rats. Br J Pharmacol 110: 50–53

Yoshida M, Mizoguchi K, Yokoo H, Tanaka T, Ishii H, Emoto H, Koga C, Tanaka M (1993) Muscimol-induced increase in dopamine release and metabolism is not observed in kainic acid-lesioned striatum of conscious rats: an in vivo microdialysis study. Neurosci Lett 162: 5–8

Zetterström T, Sharp T, Collin AK, Ungerstedt U (1988) *In vivo* measurement of extracellular dopamine and DOPAC in rat striatum after various dopamine-releasing drugs; implications for the origin of extracellular DOPAC. Eur J Pharmacol 148: 327–334

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