ELSEVIER

European Journal of Pharmacology 376 (1999) 273–278

ejp

www.elsevier.nl/locate/ejphar

# Taurine release in the rat vas deferens is modulated by $Ca^{2+}$ and is independent of contractions

Carmen Diniz, Paula da Cruz Fresco, Jorge Gonçalves *

*Laboratório de Farmacologia, CEQOFF / FCT, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha 164, P-4050 Porto, Portugal*

Received 11 February 1999; received in revised form 18 May 1999; accepted 21 May 1999

**Abstract**

Electrical field stimulation induces taurine release in rat vas deferens. In the present study, it was investigated if this release is secondary to contraction. The influence of $Ca^{2+}$ and of the stimulation conditions was also studied. Contractions evoked by electrical field stimulation (5 Hz/270 pulses, transverse or longitudinal) were recorded and released taurine was quantified by high performance liquid chromatography with fluorimetric detection. $Ca^{2+}$ removal abolished contractions, but not the overflow of taurine. Overflow elicited by longitudinal electrical field stimulation was higher than that elicited by transverse electrical field stimulation. Increasing the current strength also increased taurine overflow. In $Ca^{2+}$-free medium, taurine overflow was decreased by caffeine (5 mM) or ryanodine (10 μM) but increased by dantrolene (50 μM). The results indicate that taurine release evoked by electrical field stimulation is (i) independent of contraction, (ii) modulated by $Ca^{2+}$, (iii) potential dependent, and may be due to a decrease in taurine affinity for the plasma membrane and/or to an increase of $Na^{+}$-dependent outward transport. 

*Keywords:* Taurine release; Vas deferens; $Ca^{2+}$; Caffeine; Ryanodine; Dantrolene

## 1. Introduction

Taurine (2-aminoethane sulfonic acid) is an ubiquitous amino acid. Taurine is present in muscles and in other excitable tissues (Jacobsen and Smith, 1968), and it may reach concentrations of μmol/g tissue wet weight, accounting for up to 60% of the total free amino acid pool (Huxtable, 1992).

Release of taurine into the extracellular space can be elicited by several stimuli such as membrane depolarisation, activation of some neurotransmitter receptors, decrease in the medium osmolarity or stretching of the plasma membrane (see Shain and Martin, 1990; Huxtable, 1992). The role of $Ca^{2+}$ on taurine release has been difficult to establish. $Ca^{2+}$ is not critical for taurine release as it is in exocytotic mechanisms (see Shain and Martin, 1990). However, depending on the intensity of stimulation, removal of $Ca^{2+}$ from the medium may decrease or increase taurine overflow (Philibert et al., 1989; Queiroz et al., 1995), which suggests a role for $Ca^{2+}$ as modulator of taurine release.

It was reported previously that, in the rat vas deferens, electrical field stimulation elicits taurine release (Queiroz et al., 1995), an effect ascribed mainly to activation of postjunctional purinoceptors. However, in this study, some questions were not fully addressed: (i) contractions were not measured and, therefore, it was not possible to clarify if taurine release was secondary to the contractile response and (ii) $Ca^{2+}$ removal from the medium caused opposite effects on taurine release depending on the intensity of the stimulation and, therefore, it was not possible to clarify the role of $Ca^{2+}$ on taurine release in rat vas deferens.

In the present study, we investigated (i) if taurine release elicited by electrical field stimulation in the rat vas deferens is secondary to contraction, by measuring contractions and taurine overflow, simultaneously, and (ii) the role of $Ca^{2+}$, by removing $Ca^{2+}$ from the medium and by using drugs known to influence the release and uptake of intracellular $Ca^{2+}$. Preliminary accounts of this work have been presented elsewhere (Diniz et al., 1997a,b).

* Corresponding author. Tel.: +351-2-2078932; telefax: +351-2-2078969; E-mail: jorgemmg@mail.ff.up.pt

0014-2999/99/$ - see front matter 
PII: S0014-2999(99)00388-X

## 2. Materials and methods

Male Wistar rats (250–335 g; Instituto Gulbenkian de Ciência, Oeiras, Portugal) were killed by cervical dislocation and exsanguination. The vasa deferentia (about 55 mg and 2 cm long) were dissected and desheathed. Each vas deferens was mounted in a superfusion chamber, with one end fixed and the other attached to an isometric force transducer (MLT050, ADInstruments, East Sussex, UK), and superfused at a flow rate of 2 ml/min. The tension was recorded on a Macintosh LC computer connected to a MacLab system (ML740 with ML110 bridge amplifiers, ADInstruments). The initial tension applied was 17 mN, but tissues subsequently relaxed to approximately 8 mN. The $Ca^{2+}$-free medium contained (mM): NaCl 121.1, KCl 4.7, $KH_2PO_4$ 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, glucose 10.0, ascorbic acid 0.57, disodium EDTA 0.03; it was kept at 37°C and saturated with 95% $O_2$ and 5% $CO_2$. In the $Ca^{2+}$-containing medium, 2.5 mM $CaCl_2$ was also added, prepared by equimolar replacement of NaCl by $CaCl_2$.

A Coulbourn stimulator (E13-65, Coulbourn Instruments, Allentown, PA, USA), operating in a constant current mode, was used for electrical field stimulation. Trains of 270 electrical pulses (5 Hz, 0.5–2 ms pulse width, 10 or 60 mA) were applied by means of two platinum electrodes placed parallel to (for transverse electrical field stimulation) or at each end of the tissue (for longitudinal electrical field stimulation). In some experiments, only one stimulation period ($S_1$) was applied, starting at $t = 45$ min ($t = 0$ min was the onset of superfusion). In experiments with drugs, two stimulation periods were applied, the second ($S_2$) starting at $t = 90$ min. Drugs (or solvent) were added to the medium at $t = 55$ min and were present throughout the experiment. One-min samples of superfusion medium were collected (five samples for each stimulation period, the first sample before and the others after the onset of stimulation). At the end of the experiment, tissues were weighed and, in some tissues, the taurine content was measured. For measurements of taurine content, preparations were homogenised in sulfosalicylic acid (5 ml; 0.2 mM; 4°C), centrifuged and filtered through a 0.2 μm filter. Taurine present in superfusates and supernatants was measured by high performance liquid chromatography with fluorimetric detection as previously described (Queiroz et al., 1995).

Taurine content is expressed as μmol of taurine per gram of tissue (μmol/g). Basal outflow of taurine is expressed as nmol/g. Taurine overflow was calculated as the difference between the total taurine outflow in the 4 min after electrical field stimulation onset and the estimated basal outflow during this time (the basal outflow was assumed to be identical to the outflow before electrical field stimulation onset) and is expressed as nmol/g tissue. Ratios of taurine overflow elicited by $S_2$ and by $S_1$ ($S_2/S_1$) were calculated for further evaluation of the effects of drugs (or solvent). $S_2/S_1$ ratios obtained in individual experiments with the test compound were calculated as a percentage of the mean $S_2/S_1$ ratio in the appropriate control group.

The following drugs were used: caffeine anhydrous, dantrolene sodium salt and verapamil hydrochloride, purchased from Sigma (Alcobendas, Spain); ryanodine, from RBI (Natick, MA, USA). Solutions of drugs were prepared with dimethylsulphoxide (DMSO) and diluted with medium immediately before use. Solvent was added to the superfusion medium in parallel control experiments.

Results are expressed as arithmetic means ± S.E.M. for the number ($n$) of tissues indicated. Differences between means were tested for significance using Student's unpaired $t$-test and those with $P$ values of 0.05 or less were considered significant.

## 3. Results

Basal outflow from tissues superfused with $Ca^{2+}$-containing medium was 4.4 ± 0.9 nmol/g tissue ($n = 14$) and from tissues superfused with $Ca^{2+}$-free medium, it was 5.1 ± 0.4 nmol/g tissue ($n = 93$).

### 3.1. Influence of $Ca^{2+}$ on electrical field stimulation-evoked taurine overflow and contractions

Electrical field stimulation-evoked taurine overflow in tissues superfused with $Ca^{2+}$-containing or $Ca^{2+}$-free medium (see columns of Figs. 1 and 2), with a similar time

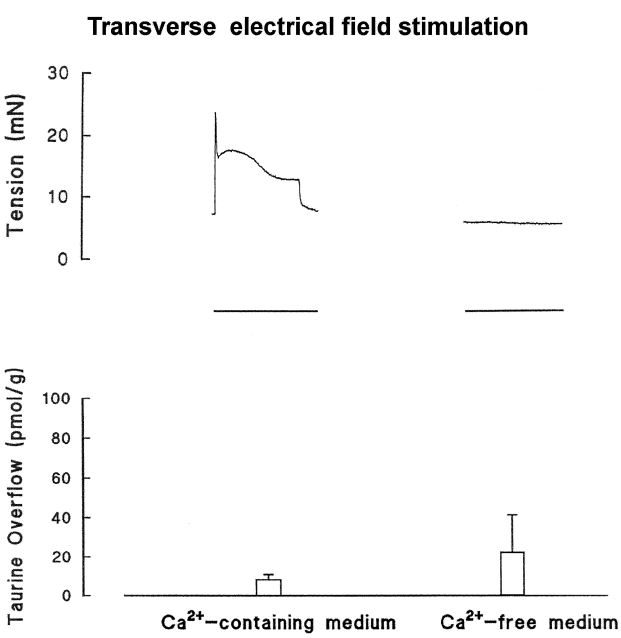


Fig. 1. Rat vas deferens/transverse electrical field stimulation: contractions (upper panel) and taurine overflow (lower panel) evoked by electrical field stimulation in $Ca^{2+}$-containing (left panel) and in $Ca^{2+}$-free medium (right panel). Tissues were stimulated once (5 Hz, 2 ms, 60 mA, 270 pulses; horizontal bar). Upper panel shows tracings of contractions from representative experiments. Lower panel shows taurine overflow (mean ± S.E.M. of four to six experiments).

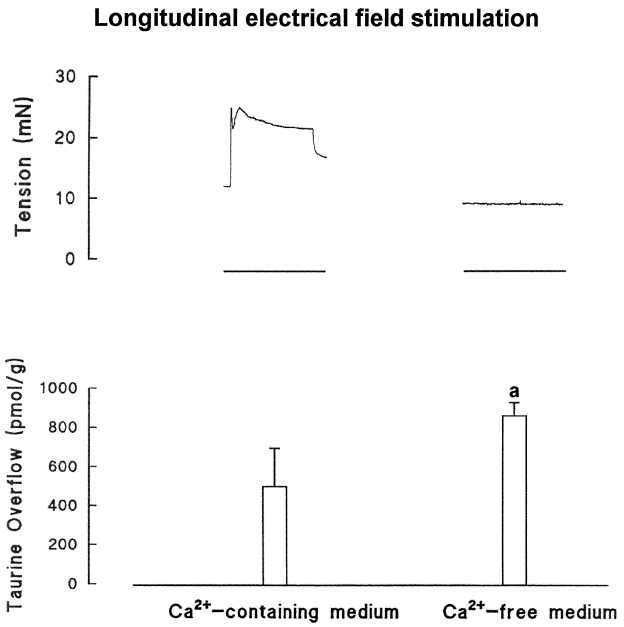


Fig. 2. Rat vas deferens/longitudinal electrical field stimulation: contractions (upper panel) and taurine overflow (lower panel) evoked by electrical field stimulation in $Ca^{2+}$-containing (left panel) and in $Ca^{2+}$-free medium (right panel). Tissues were stimulated once (5 Hz, 2 ms, 60 mA, 270 pulses; horizontal bar). Upper panel shows tracings of contractions from representative experiments. Lower panel shows taurine overflow (different scale from Fig. 1; mean ± S.E.M. of four to six experiments). Significant differences from overflow in $Ca^{2+}$-containing medium: [a] $P < 0.05$ (unpaired Student's *t*-test).

course (peaking at the third collection period, returning to values closer to those before electrical field stimulation, afterwards; not shown). Furthermore, in tissues superfused with $Ca^{2+}$-free medium, taurine overflow was higher (about 1.7 times; see Fig. 2 for longitudinal electrical field stimulation) or tended to be higher (about 2.7 times; see Fig. 1 for transverse electrical field stimulation; $P = 0.206$) than that in tissues superfused with $Ca^{2+}$-containing medium. However, both longitudinal and transverse electrical field stimulation elicited contractions but only in tissues superfused with $Ca^{2+}$-containing medium (see tracings on Figs. 1 and 2).

### *3.2. Influence of stimulation parameters on electrical field stimulation-evoked contractions and taurine overflow*

In experiments carried out with $Ca^{2+}$-containing medium, transverse and longitudinal electrical field stimulation (5 Hz, 2 ms, 60 mA, 270 pulses) elicited similar contractions (see Figs. 1 and 2, left side). However, taurine overflow elicited by longitudinal electrical field stimulation was about 63 times higher than that elicited by transverse electrical field stimulation (compare left side columns of Figs. 2 and 1). In experiments carried out with $Ca^{2+}$-free medium, longitudinal electrical field stimulation also evoked a much higher (about 38 times) taurine release than transverse electrical field stimulation did (compare right side columns of Figs. 2 and 1).

To investigate the influence of stimulation conditions on the electrical field stimulation-evoked taurine overflow, experiments were carried out with $Ca^{2+}$-free medium, using longitudinal electrical field stimulation with different current strengths (60 and 10 mA) and different pulse widths (2, 1 or 0.5 ms). A decrease in the current strength from 60 to 10 mA (pulse width of 2 ms) or a decrease in the pulse width from 2 to 0.5 ms (current strength of 60 mA) caused a marked decrease in the electrical field stimulation-evoked taurine overflow (12 and 1.7 times, respectively; Table 1).

The reproducibility of the electrical field stimulation-evoked taurine overflow was studied by comparing taurine overflow elicited by two ($S_1$ and $S_2$) identical longitudinal electrical field stimulation trains (5 Hz, 2 ms, 60 mA, 270 pulses) 45 min apart. In experiments with $Ca^{2+}$-containing medium, both $S_1$ and $S_2$ elicited contractions and taurine release. However, even though the contractions elicited by the two trains were similar (not shown), taurine overflow elicited by $S_2$ was lower than that elicited by $S_1$: $S_2/S_1$ ratio of $0.33 \pm 0.05$ ($n = 5$; $P < 0.01$). A similar decrease in taurine overflow elicited by $S_2$ was observed in experiments with $Ca^{2+}$-free medium: $S_2/S_1$ ratio $0.27 \pm 0.05$ ($n = 5$; $P < 0.01$). The decrease in the taurine overflow elicited by $S_2$ was dependent on the parameters of stimulation: the $S_2/S_1$ ratio of taurine release elicited by trains of 0.5 ms pulses (other parameters constant; longitudinal electrical field stimulation) was $0.58 \pm 0.06$ ($n = 6$), higher than the ratio obtained with trains of 2 ms pulses ($P < 0.05$).

The possibility that the decrease in $S_2/S_1$ ratio could be due to a loss of taurine tissue content was also investigated. Paired experiments where vasa deferentia were submitted to one or two stimulation periods ($S_1$ or $S_1$ and $S_2$; 5 Hz, 2 ms, 60 mA, 270 pulses; longitudinal electrical field stimulation) were carried out and the taurine content was measured at the end of the experiment (after the same time of superfusion). The taurine content of vasa deferentia

Table 1
Effect of stimulation conditions on taurine overflow elicited by longitudinal electrical field stimulation. Experiments were carried out with $Ca^{2+}$-free medium. Tissues were stimulated once (5 Hz, 270 pulses, current strength and pulse width as indicated). Shown are means ± S.E.M. of *n* experiments. Significant differences from overflow elicited by 10 mA/2 ms

| Current strength/ pulse width | Taurine overflow (nmol/g of tissue) | *n* |
|---|---|---|
| 60 mA/2 ms | 874.9 ± 28.7[a] | 6 |
| 60 mA/1 ms | 715.0 ± 95.1[a] | 4 |
| 60 mA/0.5 ms | 523.1 ± 36.7[a,b] | 5 |
| 10 mA/2 ms | 72.0 ± 27.4[b] | 5 |

[a] $P < 0.01$; from overflow elicited by 60 mA/2 ms.
[b] $P < 0.01$ (unpaired Student's *t*-test).

Table 2
Effects of drugs on electrically-evoked taurine overflow. Tissues were stimulated twice (5 Hz, 0.5 ms, 60 mA, 270 pulses; $S_1-S_2$). The drugs indicated or their solvents, were added 45 min before $S_2$; $S_2/S_1$ values are expressed as a percentage of the respective control (solvent). Means ± S.E.M. of $n$ experiments. Significant differences from control

| Drug added before $S_2$ | $S_2/S_1$ (% of control) | $n$ |
|---|---|---|
| Caffeine (5 mM) | $85 \pm 3^a$ | 5 |
| Ryanodine (10 μM) | $57 \pm 1^b$ | 5 |
| Dantrolene (50 μM) | $120 \pm 5^a$ | 5 |

[a] $P < 0.05$.
[b] $P < 0.01$ (unpaired Student's $t$-test).

submitted only to $S_1$ and to $S_1$ and $S_2$ was similar ($10.4 \pm 1.5$ and $8.9 \pm 0.8$ μmol/g tissue, respectively; $n = 5$) and much higher (about 1180 times) than the overflow elicited by each train of electrical field stimulation.

### 3.3. Influence of drugs on electrical field stimulation-evoked taurine overflow

The possibility that taurine overflow may be modulated by changes in intracellular $Ca^{2+}$ was investigated by using drugs known to interfere with $Ca^{2+}$ release from intracellular stores and with $Ca^{2+}$ binding. Experiments were carried out in $Ca^{2+}$-free medium. Tissues were stimulated twice ($S_1$ and $S_2$) with identical trains of electrical field stimulation (5 Hz, 0.5 ms, 60 mA, 270 pulses; longitudinal electrical field stimulation) and drugs were added after $S_1$.

Caffeine (5 mM) and ryanodine (10 μM), which cause the release of intracellular $Ca^{2+}$, decreased the electrical field stimulation-evoked taurine overflow (Table 2). The opposite occurred with dantrolene (50 μM), a drug that prevents $Ca^{2+}$ release (Table 2).

The effects of verapamil, an inhibitor of L-type $Ca^{2+}$ channels which also interferes with $Ca^{2+}$ binding to the plasma membrane (Chovan et al., 1980), on the electrical field stimulation-evoked taurine overflow was also investigated. Experiments were carried out in $Ca^{2+}$-free medium, and verapamil (100 μM) caused a marked increase $155 \pm 7\%$ ($n = 5$) in taurine release ($P < 0.05$).

## 4. Discussion

The term release is often used to denote the overflow of a compound from a tissue or other cellular preparation produced by an exocytotic mechanism. It is also used in a more general sense to denote the overflow independent of the mechanism involved. In the present study, the term release is used with the latter meaning.

Our results confirm previous observations of the occurrence of an electrically-evoked release of taurine in rat vas deferens (Queiroz et al., 1995). In this tissue, taurine is present in the micromolar range per gram of tissue (Queiroz et al., 1995), a fact confirmed in the present study.

Taurine release evoked by electrical field stimulation does not seem to be due to a taurine leakage from the cell since the amount of taurine released was not proportional to the taurine tissue content: a second application of an identical train of pulses elicited a much lower release of taurine than that elicited by the first stimulation, in spite of the taurine tissue content being similar.

The chambers used in the present study allowed electrical stimulation with different orientations of the electric field (longitudinal and transverse), the simultaneous measurement of contractions and the collection of superfusate samples (for further taurine measurements). Evidence obtained in the present study shows that although electrical field stimulation elicited both contractions and taurine release, contractions were not the cause of taurine release: (i) longitudinal and transverse electrical field stimulation-evoked contractions of similar magnitude but taurine release elicited by longitudinal electrical field stimulation was much greater than the release elicited by transverse electrical field stimulation and (ii) removal of $Ca^{2+}$ from the superfusion medium abolished electrical field stimulation-evoked contractions but not taurine release.

As presented above, the amount of taurine released changed markedly with the orientation of the electric field: longitudinal electrical field stimulation was more effective in evoking taurine release than transverse electrical field stimulation. Possible reasons for this different effectiveness may be related with tissue resistivity, which differs according to the orientation of the electric field: the longitudinal resistivity can be 10 times lower and, consequently, the voltage gradient through the tissue is higher in a longitudinal field (Sperelakis, 1962, 1975). Therefore, the release of taurine may occur through a voltage-dependent mechanism, with the amount of taurine released being proportional to the stimulus intensity. The increase in taurine release with increasing current strengths or pulse widths observed in the present study also supports this hypothesis. In our previous work (Queiroz et al., 1995), in addition to electrical field stimulation, exogenous agonists (noradrenaline and α,β-methylene-ATP) also evoked taurine release. Noradrenaline and α,β-methylene-ATP cause depolarisation of smooth muscle cells (Blakeley et al., 1981; Sneddon and Burnstock, 1984a,b; Allcorn et al., 1985), suggesting that a putative voltage-dependent mechanism involved in taurine release may be activated not only by electrical field stimulation but also by other depolarising stimuli.

The $Ca^{2+}$ dependence of taurine release has been difficult to establish. In some studies, taurine release was reduced (or delayed), but not abolished, by using $Ca^{2+}$-free medium and EGTA (Collins and Topiwala, 1974; Menendez et al., 1993) while in others, these procedures led to an increase in taurine release or had no effect (Korpi and Oja, 1984; Holopainen et al., 1985; Martin et al., 1989; Lombardini, 1993). Therefore, $Ca^{2+}$ dependence may differ among tissues, with the methods used to elicit release and

also with the intensity of stimulation. In primary astrocyte cultures, the $K^+$-evoked taurine release showed a $Ca^{2+}$ dependence that disappeared when $K^+$ levels were higher than 40 mM (Philibert et al., 1989). In rat vas deferens, the electrical field stimulation-evoked taurine release showed a $Ca^{2+}$ dependence that disappeared when the current strength was increased from 10 mA to 40 mA (Queiroz et al., 1995). The loss of $Ca^{2+}$ dependence with the increase in stimulus intensity suggests that taurine is released from a pool that depends on $Ca^{2+}$ for its stabilisation. A lack of $Ca^{2+}$ would decrease taurine affinity and increase its release from this pool. This putative role of $Ca^{2+}$ as a stabiliser of the releasable taurine pool is supported by the increase in taurine release seen after $Ca^{2+}$ removal and by the effects of caffeine, ryanodine and dantrolene, observed in the present study. Caffeine and ryanodine, drugs used to evoke the release of intracellular $Ca^{2+}$ in several tissues (Waterhouse et al., 1987; Bültmann et al., 1993; Ehrlich et al., 1994), decreased electrically-evoked taurine release. Dantrolene, a drug used to inhibit the release of intracellular $Ca^{2+}$ (Ohta et al., 1990), caused the opposite effect. These results further substantiate the hypothesis that $Ca^{2+}$ released from intracellular stores may also participate in the stabilisation of the releasable taurine pool, and this influence of $Ca^{2+}$ may constitute another aspect of the already known taurine/$Ca^{2+}$ interactions (see Schaffer et al., 1990; Huxtable, 1992; Namba et al., 1992).

To our knowledge, there is no evidence for the existence of separate pools of intracellular taurine, namely intravesicular taurine. However, it has been shown that taurine binds to membrane phospholipids, particularly neutral phospholipids (Huxtable and Sebring, 1986; Sebring and Huxtable, 1986; Huxtable, 1989, 1992), and that this binding is inhibited by the ionic changes occurring during stimulation, namely $Na^+$ influx (Huxtable, 1992; Quesada et al., 1992), and by drugs such as verapamil (Chovan et al., 1980). An inhibition of taurine binding may explain the observed increase in taurine release caused by verapamil.

It has been shown that taurine release is sensitive to tetrodotoxin (Della Corte et al., 1990; Queiroz et al., 1995), suggesting that taurine release is $Na^+$-dependent. Furthermore, the existence of a $Na^+$-dependent taurine transporter has been proved by cloning experiments with several tissues (rat brain: Smith et al., 1992; human thyroid cells: Jhiang et al., 1993; human placenta: Ramamoorthy et al., 1994) and cell lines (Madin–Darby canine kidney cells: Uchida et al., 1992). Therefore, the intracellular accumulation of $Na^+$ during depolarisation may not only inhibit taurine binding to the membrane, as explained above, but may also favour a $Na^+$/taurine outward transport and explain the voltage dependence of taurine release.

In conclusion, the present study shows that, in rat vas deferens, taurine release elicited by electrical field stimulation is independent of contraction, modulated by $Ca^{2+}$, and probably, potential dependent. It is proposed that taurine release evoked by electrical field stimulation may be a consequence of a decreased affinity of taurine for the plasma membrane and/or of an increase in $Na^+$/taurine outward transport.

## Acknowledgements

The authors gratefully acknowledge the technical assistance of M. Céu Pereira. This study was supported by FCT/PRAXIS XXI and JNICT/PRAXIS XXI/BM/6734/95.

## References

Allcorn, R.J., Cunnane, T.C., Kirkpatrick, K., 1985. Actions of α,β-methylene-ATP and 6-hydroxydopamine on sympathetic neurotransmission in the vas deferens of the guinea pig, rat and mouse: support for co-transmission. Br. J. Pharmacol. 89, 647–659.

Blakeley, A.G.H., Brown, D.A., Cunnane, T.C., French, A.M., McGrath, J.C., Scott, N.C., 1981. Effects of nifedipine on electrical and mechanical responses of rat and guinea pig vas deferens. Nature 294, 759–761.

Bültmann, R., von Kügelgen, I., Starke, K., 1993. Effects of nifedipine and ryanodine on adrenergic neurogenic contractions of rat vas deferens: evidence for a pulse-to-pulse change in $Ca^{2+}$ sources. Br. J. Pharmacol. 108, 1062–1070.

Chovan, J.P., Kulakowski, E.C., Sheakowski, S., Schaffer, S.W., 1980. Calcium regulation by the low-affinity taurine binding sites of cardiac sarcolemma. Mol. Pharmacol. 17, 295–300.

Collins, G.C.S., Topiwala, S.H., 1974. The release of $^{14}$C-taurine from slices of rat cerebral cortex and spinal cord evoked by electrical stimulation and high potassium ion concentrations. Br. J. Pharmacol. 50, 451P–452P.

Della Corte, L., Bolam, J.P., Clarke, D.J., Parry, D.M., Smith, A.D., 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.

Diniz, C., Fresco, P., Gonçalves, J., 1997a. Taurine release in rat vas deferens is dependent on stimulation conditions but is not secondary to contractions. Pharmacol. Toxicol. 81, 42.

Diniz, C., Fresco, P., Gonçalves, J., 1997b. Calcium inhibits the electrically-evoked taurine release in rat vas deferens. Pharmacol. Toxicol. 81, 42.

Ehrlich, B.E., Kaftan, E., Bezprozvannaya, S., Bezprozvannya, I., 1994. The pharmacology of intracellular $Ca^{2+}$-release channels. Trends Pharmacol. Sci. 15, 145–149.

Holopainen, I., Kontro, P., Oja, S.S., 1985. Release of preloaded taurine and hypotaurine from astrocytes in primary culture: stimulation by calcium-free media. Neurochem. Res. 10, 123–130.

Huxtable, R.J., 1989. Taurine in the central nervous system and the mammalian actions of taurine. Prog. Neurobiol. 32, 471–533.

Huxtable, R.J., 1992. Physiological actions of taurine. Physiol. Rev. 72, 101–163.

Huxtable, R.J., Sebring, L.A., 1986. Towards a unifying theory for the actions of taurine. Trends Pharmacol. Sci. 7, 481–485.

Jacobsen, J.G., Smith, L.H., 1968. Biochemistry and physiology of taurine and taurine derivatives. Physiol. Rev. 48, 424–511.

Jhiang, S.M., Fithian, L., Smanik, P., McGill, J., Tong, Q., Mazzaferri, E.L., 1993. Cloning of the human taurine transporter and characterization of taurine uptake in thyroid cells. FEBS Lett. 318, 139–144.

Korpi, E.R., Oja, S.S., 1984. Calcium chelators enhance the efflux of taurine from brain slices. Neuropharmacology 23, 377–380.

Lombardini, J.B., 1993. Spontaneous and evoked release of [$^3$H] taurine from a $P_2$ subcellular fraction of the rat retina. Neurochem. Res. 18, 193–202.

Martin, D.L., Madelian, V., Shain, W., 1989. Spontaneous and beta-adrenergic receptor-mediated taurine release from astroglial cells do not require extracellular calcium. J. Neurosci. Res. 23, 191–197.

Menendez, N., Solís, J.M., Herreras, O., Galarreta, M., Conojero, C., Martín del Rio, R., 1993. Taurine release evoked by NMDA receptor activation is largely dependent on calcium mobilization from intracellular stores. Eur. J. Neurosci. 5, 1273–1279.

Namba, K., Ejiri, K., Kanemori, H., Kudo, T., Sekiba, K., 1992. Effect of taurine concentration on platelet aggregation in gestosis patients with edema, proteinuria and hypertension. Acta Med. Okayama 46, 241–247.

Ohta, T., Ito, S., Ohga, A., 1990. Inhibitory action of dantrolene on Ca-induced $Ca^{2+}$ release from sarcoplasmic reticulum in guinea pig skeletal muscle. Eur. J. Pharmacol. 178, 11–19.

Philibert, R.A., Rogers, K.L., Dutton, G.R., 1989. $K^+$-evoked taurine efflux from cerebellar astrocytes: on the roles of $Ca^{2+}$ and $Na^+$. Neurochem. Res. 14, 43–48.

Queiroz, G., Gonçalves, J., Carvalho, F., Vale, P., 1995. Electrically-evoked release of taurine in the rat vas deferens: evidence for a purinoceptor-mediated effect. Naunyn-Schmiedeberg's Arch. Pharmacol. 351, 60–66.

Quesada, O., Lu, P., Sturman, J.A., 1992. Taurine distribution in the cat muscle: an immunohistochemical study. In: Lombardini, J.B., Schaffer, S.W., Azuma, J. (Eds.), Advances in Experimental Medicine and Biology, Vol. 315 (Taurine — Nutritional Value and Mechanisms of Action), Plenum, New York, NY, pp. 99–104.

Ramamoorthy, S., Leibach, F.H., Mahesh, V.B., Han, H., Yang-Feng, T., Blakely, R.D., Ganapathy, V., 1994. Functional characterization and chromosomal localization of a cloned taurine transporter from human placenta. Biochem. J. 300, 893–900.

Schaffer, S.W., Allo, S., Harada, H., Azuma, J., 1990. Regulation of calcium homeostasis by taurine: role of calmodulin. In: Pasantes-Morales, H., Martin, D.L., Shain, W., Del Rio, R.M. (Eds.), Taurine: Functional Neurochemistry, Physiology and Cardiology, Wiley-Liss, New York, NY, pp. 217–225.

Sebring, L.A., Huxtable, R.J., 1986. Low affinity binding of taurine to phospholiposomes and cardiac sarcolemma. Biochim. Biophys. Acta 884, 559–566.

Shain, W., Martin, D.L., 1990. Uptake and release of taurine: an overview. In: Pasantes-Morales, H., Martin, D.L., Shain, W., Martín Del Rio, R. (Eds.), Taurine: Functional Neurochemistry, Physiology and Cardiology, Wiley-Liss, New York, NY, pp. 243–252.

Smith, K.E., Borden, L.A., Wang, C.H., Hartig, P.R., Branchek, T.A., Weinshank, R.L., 1992. Cloning and expression of a high affinity taurine transporter from rat brain. Mol. Pharmacol. 42, 563–569.

Sneddon, P., Burnstock, G., 1984a. Inhibition of excitatory junction potentials in guinea-pig vas deferens by $\alpha,\beta$-methylene-ATP: further evidence for ATP and noradrenaline as co-transmitters. Eur. J. Pharmacol. 100, 85–90.

Sneddon, P., Burnstock, G., 1984b. ATP as a co-transmitter in rat tail artery. Eur. J. Pharmacol. 106, 149–152.

Sperelakis, N., 1962. Contraction of depolarized smooth muscle by electric fields. Am. J. Physiol. 202, 731–742.

Sperelakis, N., 1975. Electrical stimulation of muscle: field stimulation. In: Daniel, E.E., Paton, D.M. (Eds.), Methods in Pharmacology, Vol. 3 (Smooth muscle), Plenum, London, pp. 321–337.

Uchida, S., Kwon, H.M., Yamauchi, A., Preston, A.S., Marumo, F., Handler, J.S., 1992. Molecular cloning of the cDNA for an MDCK cell $Na^+$- and $Cl^-$-dependent taurine transporter that is regulated by hypertonicity. Proc. Natl. Acad. Sci. U.S.A. 89, 8230–8234.

Waterhouse, A.L., Pessah, I.N., Francini, A.O., Casida, J.E., 1987. Structural aspects of ryanodine action and selectivity. J. Med. Chem. 30, 710–716.