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Interaction between organic calcium-channel blockers and taurine in vitro and in vivo

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Taurine is a positive inotropic agent in guinea-pig auricles perfused with a normal calcium medium, it potentiates the positive inotropic effect of strophanthin-K (Guidotti et al 1971) and ouabain (Iwata & Fujimoto 1976). Taurine inhibits the decrease in contractile force due to a calcium-free medium (Dolara et al 1973) in the isolated and perfused heart. Furthermore the positive inotropic effect of taurine is more evident in vitro when the calcium concentration in the external medium is low (Dietrich & Diacono 1971; Bandinelli et al 1981). There is evidence that the potentiating effect of taurine on the positive inotropic effect of ouabain is to some extent related to an accumulation of intracellular calcium in taurine-loaded heart (Iwata & Fujimoto 1976); Dolara et al (1973) also found that taurine increased calcium levels in cardiac tissue.

On the other hand, taurine antagonized the negative inotropic effect of verapamil in rat isolated and perfused hearts (Chovan et al 1980).

We decided to examine the interaction between methoxy verapamil (D600) and taurine in guinea-pig ventricular strips, since rat and guinea-pig hearts are known to behave differently in many respects, e.g. the digitalis response and the staircase phenomenon (Allen & Schwartz 1969). The interaction between taurine and verapamil was also studied in vivo.

Materials and methods

Drugs used. methoxyverapamil hydrochloride and verapamil hydrochloride were kindly supplied by Knoll A. G. Ludwigshafen, F.R.G., taurine was obtained from Merck Darmasdt, F.R.G.

Two strips were removed from the same right ventricle of male guinea-pigs (350–400 g) and placed in two separate tissue baths holding 20 ml of the following solution (mM): NaCl 115, KCl 4·7, $MgSO_4$ 1·2, $CaCl_2$ 1·8, KH_2PO_4 1·2, $NaHCO_3$ 25 and glucose 10, aerated with 95% O_2 and 5% CO_2 and maintained at 30 °C. The solution flowed through the organ bath at a constant rate of 6 ml min^{-1}. The length of the strip was 4–5 mm and the width of the strip was about 2 mm.

The preparation was connected to a Marb force displacement transducer and the change in tension was isometrically recorded on a Marb Polygraph 450. Resting force was adjusted to and maintained at 1 g. The ventricular strips were electrically stimulated at a constant rate of 120 beats min^{-1} (2 Hz) by square wave pulse generation at a constant current. The stimulus duration was 0·2 ms and the intensity twice the usual threshold current of 0·3–0·4 mA. After 1 h of stabilization the treated strips were exposed to taurine and methoxyverapamil, and the control to methoxyverapamil and sucrose at equimolar concentration with taurine. After the end of superfusion the strips were wiped and frozen. The calcium in the strips was measured by atomic absorption according to Dolara et al (1973).

Extracellular water was determined by [^{3}H]inulin (specific activity 1·21 mC $mmol^{-1}$; Radiochemical Centre, Amersham) according to Ross & Mokotoff (1951); total water was determined by subtracting dry weight from wet weight; intracellular water by the difference between total water and extracellular water.

The effect on frequency was studied on spontaneously-beating guinea-pig atria maintained in the conditions described for ventricular strips.

Fourteen groups of 25 male mice each weighing approximately 25 g were used for LD50 determination. Seven groups were treated with taurine 4 g kg^{-1} day^{-1} i.p. for 3 days. On the third day the control and taurine-treated groups received verapamil s.c. in doses varying from 25 mg to 150 mg kg^{-1}. The LD50 was calculated according to Finney (1964).

Results

The inotropic response to taurine and methoxyverapamil was followed for 1 h, the decrease in contraction induced by the drug in ventricular strips is shown in Fig. 1; this reached the maximum effect in 10 min and remained stable for at least 1 h. In the presence of taurine the decrease in contractility was less evident and after 10 min the force of contraction began to increase again; reaching control value after 45 min with 20 mM taurine. The taurine effect is dose-dependent as shown in Fig. 1.

The effect of different treatments on the calcium level in ventricular strips is shown in Table 1. Methoxyverapamil alone decreased the level of this ion in a statistically significant manner, while 20 mM taurine increased the level

Table 1. Effect of taurine on calcium levels (mequiv $litre^{-1}$ of intracellular water) in guinea-pig ventricular strips.

Control	20 mM taurine	10^{-7} g $litre^{-1}$ MV	10^{-7} g $litre^{-1}$ MV + 20 mM taurine
13·1 ± 1·04 (25)	17·91 ± 3·91* (8)	10·57 ± 1·53** (7)	14·37 ± 1·06*** (7)

MV = methoxyverapamil.
Values are mean ± s.e.: number of experiments in brackets.
* $P \leq 0.02$ versus control.
** $P \leq 0.001$ versus control.
*** $P \leq 0.001$ versus D600 10^{-7} g $litre^{-1}$.

* Correspondence.

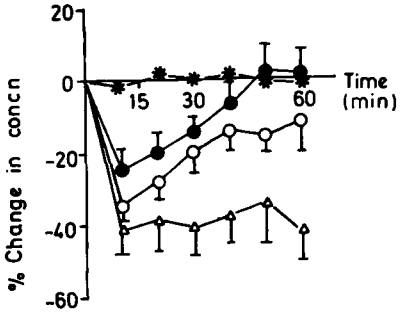

FIG. 1. Effect of methoxyverapamil and taurine on contraction in guinea-pig ventricular strips. Results are mean ± s.e. of six experiments. * without treatment; △ Drug 10^{-7} g litre^{-1}; ○ drug 10^{-7} g litre^{-1} + taurine 10 mM; ● Drug 10^{-7} g litre^{-1} + taurine 20 mM.

of this cation in cardiac tissue; when methoxyverapamil and taurine were administered together, the calcium level found in cardiac tissue was similar to that measured in the control strips.

Taurine given alone slightly decreased spontaneous atrial rate but when it was administered together with methoxyverapamil it prevented the negative chronotropic effect of the calcium antagonist (Table 2).

In vivo the i.p. administration in the mouse of taurine 4 g kg^{-1} day^{-1} for 3 days increased the LD50 of verapamil from 30·77 to 63·15 mg kg^{-1} (Table 3). The confidence limits in verapamil-treated mice were between 25·5–37·0 mg kg^{-1} while in taurine- and verapamil-treated animals the confidence limits were 57·7–68·9. Limits were calculated for 95% of probability.

Discussion

The present study shows that taurine inhibited the negative inotropic and chronotropic effect of methoxyverapamil in ventricular strips and atria respectively.

The positive inotropic effect of taurine could be a consequence of the increase in cardiac calcium levels. Numerous findings have shown that cardiac tissue accumulates calcium under conditions of increased contractility (Nayler & Merrillees 1971). Dolara et al (1976) have shown that taurine increases both the rate of calcium binding and total calcium accumulation by sarcoplasmic reticulum isolated from guinea-pig heart. These authors suggest that the positive inotropic effect can be correlated to an increased affinity of sarcoplasmic reticulum for calcium. Chovan et al (1979) found that taurine enhances calcium binding to the low affinity sites of rat cardiac sarcolemma. Taurine reverses the inhibitory effect of both verapamil and La^{3+} on calcium binding by rat sarcolemma (Chovan et al 1979) it is possible that taurine can act at the sarcolemma level. The fact that the recovery of contraction after methoxyverapamil + taurine is a slow phenomenon suggests an intracellular action of taurine.

Also in vivo taurine antagonizes the lethal effect of verapamil. The very high dose of taurine used in in vivo experiments may be justified considering the high-levels of

Table 2. Effect of methoxyverapamil (MV) and taurine on frequency in guinea-pig isolated spontaneously-beating atria.

Conditions	Beats min^{-1}
Control	111 ± 4 (23)
20 mM taurine	100 ± 4 (6)
10^{-6} g litre^{-1} MV	81 ± 4* (6)
10^{-6} g litre^{-1} MV + 20 mM taurine	97 ± 3 (6)

Results are mean ± s.e.; in brackets the number of experiments.
* $P \leq 0{\cdot}001$.

Table 3. LD50 of s.c. verapamil in mice untreated with taurine and in mice pretreated with 4 g kg^{-1} day^{-1} taurine for 3 days.

	Taurine untreated animals	Taurine pretreated animals
LD50 of verapamil (mg kg^{-1})	30·77	63·15
Confidence* limits	25·5–37·0	57·7–68·9

* Confidence limits were calculated for 95% probability.

taurine found in cardiac and other tissues (Jacobsen & Smith 1968). The fact that taurine can shift the LD50 of verapamil positively confirms the interaction between taurine and calcium-channel blockers and also suggests that this relatively non-toxic compound, a normal constituent of human diet, could be found a use in the treatment of poisoning by verapamil or other calcium antagonists.

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REFERENCES

Allen, J. C., Schwartz, A. (1969) J. Pharmacol. Exp. Ther. 168: 42–53

Bandinelli, R., Franconi, F., Giotti, A., Martini, F., Moneti, G., Stendardi, I., Zilletti, L. (1981) Br. J. Pharmacol. 67: 115–116P

Chovan, J. P., Kulakowski, E. G., Benson, B. W., Schaffer, S. W. (1979) Biochim. Biophys. Acta 551: 129–136

Chovan, J. P., Kulakowski, E. G., Sheakowski, S., Schaffer, S. W. (1980) Mol. Pharmacol. 17: 295–300

Dietrich, J., Diacono, J. (1971) Life Sci 10: 499–507

Dolara, P., Agresti, A., Giotti, A., Pasquini, G. (1973) Eur. J. Pharmacol. 24: 532–538

Dolara, P., Agresti, A., Giotti, A., Sorace, E. (1976) Can. J. Physiol. Pharmacol. 54: 529–547

Finney, D. J. (1964) Statistical Methods in Biological Assay. Charles Griffin Co. Ltd, London

Guidotti, A., Badiani, G., Giotti, A. (1971) Pharmacol. Res. Commun. 3: 29–38

Iwata, H., Fujimoto, S. (1976) Experientia 32: 1559–1561

Jacobsen, J., Smith, L. H. (1968) Physiol. Rev. 48: 424–511

Nayler, W. G., Merrillees, N. C. R. (1971) Calcium and the heart. in: P. Harris, L. Opie (eds) Academic Press, London

Ross, G., Mokotoff, R. (1951) Biol. Chem. 190: 659–666