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Comparison of the cardiovascular effects of *trans*-diclofurime with different types of calcium antagonists in conscious spontaneously hypertensive rats

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1 *Trans*-diclofurime has been shown to be a potent group II calcium antagonist in *in vitro* and *in vivo* test systems. In contrast to the dihydropyridines, group II calcium antagonists have a reduced propensity to cause reflex tachycardia due to well-balanced inhibitory effects in smooth muscle and heart. Since effects on autonomic reflexes are more reliably assessed in conscious animals, the cardiovascular effects of *trans*-diclofurime have been examined and compared to those of nifedipine, verapamil and diltiazem in the conscious spontaneously hypertensive rat (SHR).

2 Each SHR had an indwelling catheter in the femoral artery to record mean arterial pressure (MAP) and heart rate (HR) and a cannula in the femoral vein for drug infusion over 1 min.

3 Nifedipine (0.1–3.0 μmol kg^{-1} i.v.) caused dose-related falls in MAP accompanied by dose-related increases in HR. *Trans*-diclofurime and verapamil (0.3–3.0 μmol kg^{-1} i.v.) also caused dose-related decreases in MAP, but significant tachycardia was only seen at 1.0 and 3.0 μmol kg^{-1}. *Trans*-diclofurime (0.3 μmol kg^{-1}) induced a significant fall in HR. Diltiazem (1.0–10.0 μmol kg^{-1} i.v.) induced dose-related falls in MAP, significant bradycardia was evident with 1.0 μmol kg^{-1} and tachycardia with 10 μmol kg^{-1}. *Trans*-diclofurime and diltiazem induced less tachycardia than nifedipine and verapamil for equivalent falls in MAP.

4 These results suggest that *trans*-diclofurime is a potent antihypertensive agent in conscious SHR and, like diltiazem, the hypotensive effects are associated with less tachycardia than is usually apparent with calcium antagonists such as nifedipine or verapamil.

5 The cardiovascular effects of *trans*-diclofurime in conscious SHR are those expected of a class II calcium antagonist and are consistent with its proposed mode of interaction with the diltiazem site in the calcium channel.

Introduction

On the basis of functional tests, calcium antagonists can be divided into at least three distinct subgroups (Spedding, 1985a, b). Group I comprises dihydropyridines, which have marked selectivity for vascular smooth muscle, the resulting vasodilatation giving rise to a reflex mediated tachycardia. Group II consists of relatively hydrophilic basic compounds and includes verapamil, diltiazem and piprofural (Spedding, 1985b). These compounds have well balanced inhibitory effects in heart and smooth muscle and although their functional effects appear to be similar, it has been found that at least two binding sites exist for this group in the calcium channel, with differing selectives for verapamil and diltiazem (Glossman & Ferry 1983; Boles *et al.*, 1984). In contrast to group I and group II calcium antagonists, group III drugs, exemplified by the diphenylalkylamines may have multiple sites of action. These include the calcium channel (Fleckenstein, 1983; Bolger *et al.*, 1983; Godfraind & Wiko, 1985) and calmodulin (Landry *et al.*, 1981; Spedding, 1985a) as well as having non-specific effects following accumulation within cells or in the sarcolemma (Spedding, 1985a).

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The group II calcium antagonists are particularly beneficial in cardiovascular disease, due to their well-balanced inhibitory effects in heart and smooth muscle, and therefore have been shown to be superior antiarrhythmic and antianginal agents as compared to the dihydropyridines which have a propensity to cause marked reflex tachycardia (Subramanian *et al.*, 1982). However, despite the proven effectiveness of this group of calcium antagonists in the clinic there remain very few examples of potent group II calcium antagonists. *Trans*-diclofurime has been demonstrated to be a potent group II calcium antagonist both in *in vitro* and *in vivo* test systems (Spedding *et al.*, 1987), resembling verapamil and diltiazem in this respect. The fact that it has also been shown to have the same molecular site of action as diltiazem is consistent with its classification (Mir & Spedding, 1987). Moreover, the calcium antagonist effects of diclofurime were found to exhibit marked stereoselectivity both *in vitro* and *in vivo*; the *trans* isomer is more potent than the *cis* isomer, and their relative functional potencies are closely reflected in their affinities for the diltiazem binding site. The association between the affinity of compounds for the diltiazem binding site and their functional calcium antagonist potency is supported by a recent study on diltiazem and its metabolites (Schoemaker *et al.*, 1987).

With the objective of further characterizing the cardiovascular profile of diclofurime, the cardiovascular effects of *trans*-diclofurime have been further examined and compared, firstly, to those of verapamil and diltiazem and, secondly, to those of nifedipine, a representative of group I. Since effects on autonomic reflexes are most reliably assessed in conscious animals, these studies have been carried out in conscious spontaneously hypertensive rats (SHR).

Methods

Animals

Male SHR (Iffa-Credo, France) weighing 340–420 g were used. They were maintained on standard rat chow and tap water and a 12 h light and dark cycle.

Operative procedure and measurement of blood pressure and heart rate

Under ether anaesthesia, a catheter (pp 10 fused to pp 50) was inserted into the femoral artery and exteriorised at the scruff of the neck. Mean arterial pressure (MAP) and heart rate (HR) were recorded by means of a Bentley Trantec model 800 transducer attached to a Hewlett Packard recorder. Similarly a cannula (pp 10 fused to pp 50) was placed in the femoral vein and exteriorised at the scruff of the neck for drug administration intravenously (i.v.).

Dosing schedule

At least 4 h after operation, control measurements of MAP and HR were taken. Subsequently 1 ml kg^{-1} 50% polyethylene glycol 300 (PEG, for nifedipine) or 0.9%, saline, vehicle controls, was infused i.v. over a 1 min period and MAP and HR determined at various time intervals for the following 30–60 min. This was followed by the i.v. infusion of nifedipine, verapamil, *trans*-diclofurime (0.1–3.0 μmol kg^{-1}) or diltiazem (0.3–10.0 μmol kg^{-1}) over a 1 min period and MAP and HR were recorded during the following 30–60 min (90 min for nifedipine). Following the control injection, each compound was administered in a cumulative fashion and the blood pressure was allowed to return to control values before injection of subsequent doses.

Statistics

All values used in analysis represent the means ± s.d. Comparisons were performed by means of analysis of variance (ANOVA) with application of the Newman-Keuls test. Analysis of covariance was used to obtain the level of significant difference between the slopes and comparison of the confidence intervals was used to determine which slopes were different.

Results

Cardiovascular effects of trans-*disclofurime*

Trans-diclofurime, (0.3–3.0 μmol kg^{-1} i.v.) caused dose-related falls in MAP (Figure 1a). The dose estimated to lower MAP by 25 mmHg, obtained from the correlation of change in MAP versus dose, was 1.4 μmol kg^{-1} i.v. The decrease in MAP induced by the two highest doses of *trans*-diclofurime (1.0 and 3.0 μmol kg^{-1}) was associated with tachycardia (Figure 1b), whereas a slight, but significant fall in HR accompanied the small depressor effect induced by 0.3 μmol kg^{-1} (Figure 1b).

Cardiovascular effects of verapamil and diltiazem

Verapamil (0.3–3.0 μmol kg^{-1} i.v.) also induced a dose-related reduction in MAP (Figure 2a), 1.6 μmol kg^{-1} being the dose estimated to lower MAP by 25 mmHg. The fall in MAP induced by the

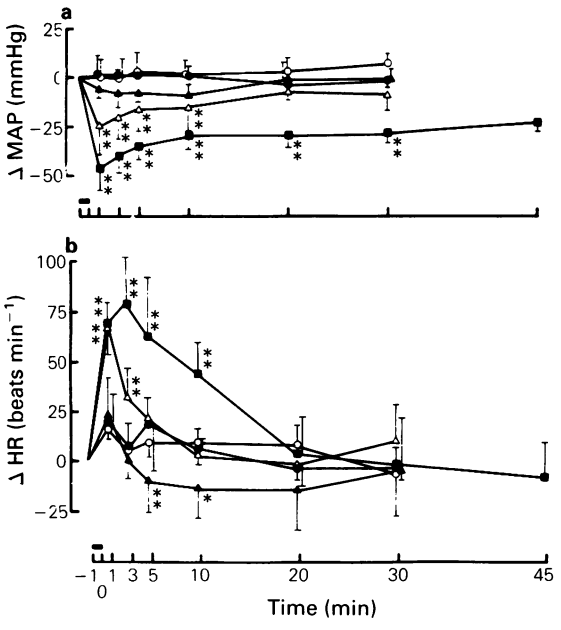

Figure 1 The change (a) in mean arterial pressure (MAP) and (b) heart rate (HR) induced by saline and increasing doses of *trans*-diclofurime. The results are expressed as the mean of 4 rats in each group (vertical lines indicate s.d.) and were compared by means of ANOVA with application of the Newman-Keuls test. $*P < 0.05$, $**P < 0.01$ when the effects of that particular dose of *trans*-diclofurime were compared to the effects of saline at the same time. (●) Saline (159 ± 10 mmHg, 305 ± 21 beats min^{-1}); *trans*-diclofurime: (○) 0.1 μmol kg^{-1} (160 ± 15 mmHg, 293 ± 37 beats min^{-1}), (▲) 0.3 μmol kg^{-1} (164 ± 13 mmHg, 290 ± 25 beats min^{-1}), (△) 1 μmol kg^{-1} (163 ± 19 mmHg, 271 ± 9 beats min^{-1}), (■) 3.0 μmol kg^{-1} (160 ± 8 mmHg, 280 ± 16 beats min^{-1}); the values in parentheses represent resting MAP and HR, respectively. The bar from −1–0 min represents an injection time of 1 min in this and the subsequent figures.

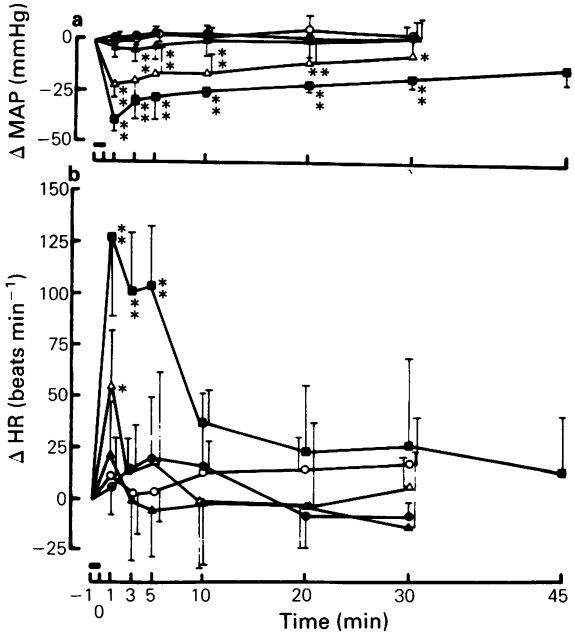

Figure 2 The change (a) in mean arterial pressure (MAP) and (b) heart rate (HR) induced by saline and increasing doses of verapamil. The results are expressed as the mean of 4 rats (vertical lines indicate s.d.) and were compared by means of ANOVA with application of the Newman-Keuls test. $*P < 0.05$, $**P < 0.01$ when the effects of that particular dose of verapamil were compared to the effects of saline at the same time. (●) Saline (151 ± 11 mmHg, 300 ± 14 beats min^{-1}); verapamil: (○) 0.1 μmol kg^{-1} (157 ± 11 mmHg, 280 ± 14 beats min^{-1}), (▲) 0.3 μmol kg^{-1} (156 ± 10 mmHg, 294 ± 19 beats min^{-1}), (△) 1.0 μmol kg^{-1} (163 ± 12 mmHg, 289 ± 28 beats min^{-1}), (■) 3.0 μmol kg^{-1} (156 ± 6 mmHg, 280 ± 8 beats min^{-1}); the values in parentheses representing MAP and HR, respectively.

two highest doses (1.0 and 3.0 μmol kg^{-1}) was accompanied by tachycardia (Figure 2b). Similarly, diltiazem (1.0–10.0 μmol kg^{-1} i.v.) caused dose-related falls in MAP (Figure 3a). Significant bradycardia was associated with a dose of 1.0 μmol kg^{-1} and tachycardia with 10 μmol kg^{-1} (Figure 3b). The dose of diltiazem estimated to reduce MAP by 25 mmHg was 6.4 μmol kg^{-1}.

Cardiovascular effects of nifedipine

The dihydropyridine nifedipine (0.1–3.0 μmol kg^{-1}) also induced dose-related depressor responses (Figure 4a), which were accompanied by dose-related increases in heart rate (Figure 4b). The dose required to lower MAP by 25 mmHg was estimated to be 0.6 μmol kg^{-1} i.v.

Regression analysis of the cardiovascular effects of the 4 calcium antagonists

The change in MAP was significantly correlated with the change in HR for each of the calcium antagonists, as demonstrated by linear regression analysis (Figure 5). Nevertheless, the slopes for verapamil and nifedipine were significantly ($P < 0.05$) greater than those of *trans*-diclofurime and diltiazem being -2.1 ± 0.3 and -1.9 ± 0.1 beats min^{-1} mmHg^{-1} as compared to -0.6 ± 0.4 and -1.2 ± 0.2 beats min^{-1} mmHg^{-1}, respectively

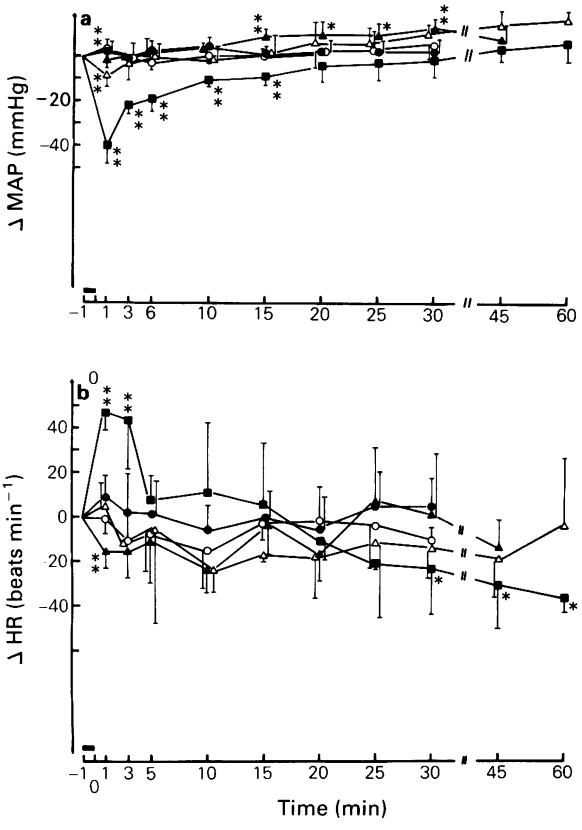

Figure 3 The change (a) in mean arterial pressure (MAP) and (b) heart rate (HR) induced by saline and increasing doses of diltiazem. The results are expressed as the mean of 4 rats (vertical lines indicate s.d.) and were compared by means of ANOVA with application of the Newman-Keuls test. $*P < 0.05$, $**P < 0.01$ when the effects of that particular dose of diltiazem were compared to the effects of saline at the same time. (●) Saline (150 ± 6 mmHg, 296 ± 17 beats min^{-1}); diltiazem: (○) 0.3 μmol kg^{-1} (151 ± 7 mmHg, 355 ± 6 beats min^{-1}), (▲) 1.0 μmol kg^{-1} (154 ± 11 mmHg, 340 ± 18 beats min^{-1}), (△) 3.0 μmol kg^{-1} (157 ± 13 mmHg, 333 ± 26 beats min^{-1}), (■) 10.0 μmol kg^{-1} (156 ± 3 mmHg, 333 ± 15 beats min^{-1}); the values in parentheses represent MAP and HR, respectively.

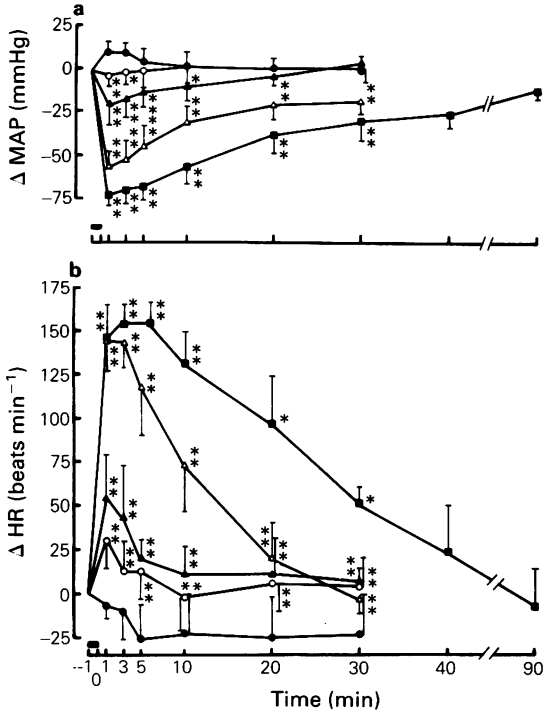

Figure 4 The change (a) in mean arterial pressure (MAP) and (b) heart rate (HR) induced by 50% polyethylene glycol 300 (PEG) and increasing doses of nifedipine. The results are expressed as the mean of 6 rats (vertical lines indicate s.d.) and were compared by means of ANOVA with application of the Newman-Keuls test. $*P < 0.05$, $**P < 0.01$ when the effects of that particular dose of nifedipine were compared to the effects of PEG at the same time. (●) 50% PEG (176 ± 7 mmHg, 315 ± 19 beats min^{-1}); nifedipine: (○) 0.1 μmol kg^{-1} (176 ± 13 mmHg, 292 ± 22 beats min^{-1}), (▲) 0.3 μmol kg^{-1} (176 ± 10 mmHg, 296 ± 16 beats min^{-1}), (△) 1.0 μmol kg^{-1} (178 ± 7 mmHg, 303 ± 20 beats min^{-1}), (■) 3.0 μmol kg^{-1} (173 ± 8 mmHg, 290 ± 23 beats min^{-1}); the values in parentheses represent MAP and HR, respectively.

(Figure 5). Hence the reflex tachycardia following verapamil and nifedipine treatment was significantly greater than that caused by either *trans*-diclofurine or diltiazem for a given fall in MAP. There was no significant difference between the slopes of verapamil and nifedipine or *trans*-diclofurime and diltiazem. No significant differences between the initial baseline values of MAP and HR were observed.

Discussion

These results demonstrate that *trans*-diclofurime is a potent antihypertensive agent upon i.v. administration in conscious SHR, being 1.1 and 4.6 times more potent than verapamil and diltiazem, respectively, but 2.3 times less potent than nifedipine in inducing a 25 mmHg fall in MAP. In addition, it has been shown that for equivalent falls in MAP, *trans*-diclofurime and diltiazem induced less reflex tachycardia than both nifedipine and verapamil.

Group I calcium antagonists such as nifedipine have a marked selectivity for vascular smooth muscle, the resulting vasodilatation giving rise to a

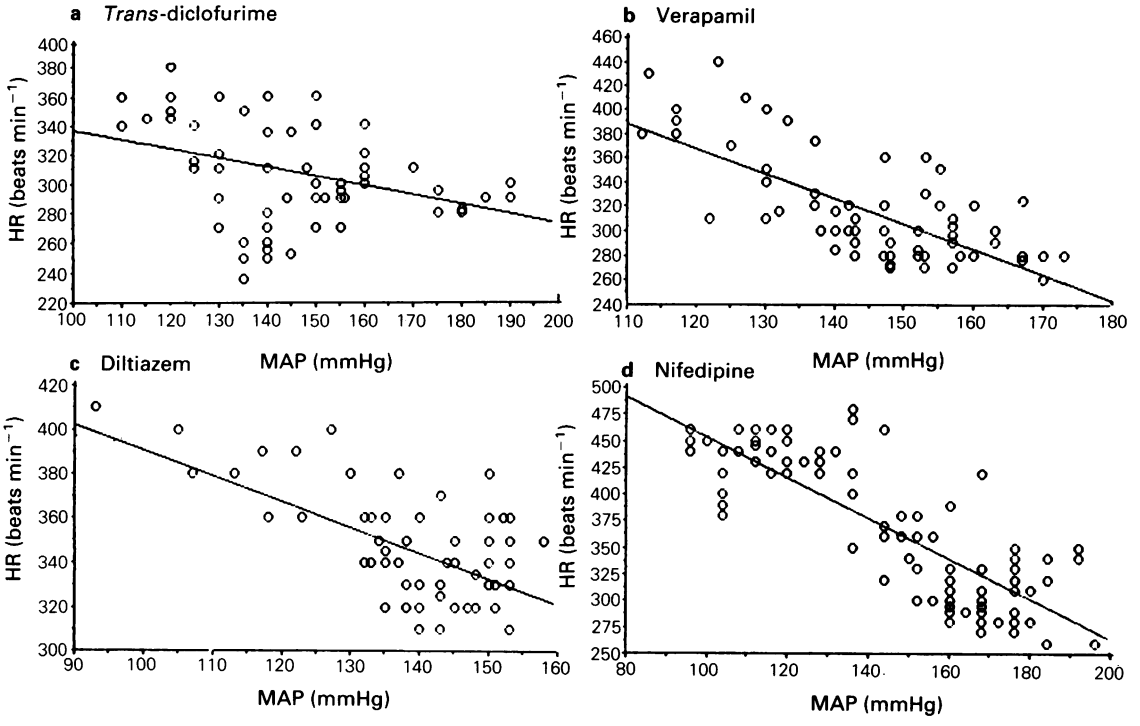

Figure 5 Regression analyses of mean arterial pressure (MAP) versus heart rate (HR) after administration of the four calcium antagonists. The regression analyses take into account the MAP and HR before and 1, 3, 5 and 10 min after administration of 4 doses of each drug $n = 4$–6. (a) *Trans*-diclofurime: slope, -0.6 ± 0.2; r (correlation coefficient) -0.4 ($P < 0.002$); 95% confidence interval, lower -1.0, upper -0.3. (b) Verapamil: slope -2.1 ± 0.3; $r - 0.7$ ($P < 0.0001$); 95% confidence interval, lower -2.6, upper -1.6. (c) Diltiazem: slope -1.2 ± 0.2; $r - 0.62$ ($P < 0.0001$); 95% confidence interval, lower -1.5, upper -0.8. (d) Nifedipine: slope -1.9 ± 0.1; $r - 0.84$ ($P < 0.0001$); 95% confidence interval, lower -2.2, upper -1.7. The slope of the lines for *trans*-diclofurime and diltiazem were significantly ($P < 0.05$) smaller than those of verapamil and nifedipine, as demonstrated by comparison of the 95% confidence intervals.

reflex mediated tachycardia in conscious animals, as demonstrated in the present study. The group II calcium antagonists, on the other hand, have been shown to have well balanced effects in heart and smooth muscle, resulting in a fall in blood pressure accompanied by direct effects on the heart (Spedding, 1982; Subramanian *et al.*, 1982; Hof, 1984), particularly causing sinus slowing or atrioventricular block, although these effects are usually readily offset by reflex adaptations. In the present study, the small fall in MAP induced by *trans*-diclofurime ($0.3\,\mu mol\,kg^{-1}$) was accompanied by a decrease in HR, which may be due to direct effects on the heart in the absence of reflex, compensatory tachycardia. Higher doses induced a fall in MAP associated with an increase in HR, although the tachycardia was not greatly augmented in response to the highest dose of $3.0\,\mu mol\,kg^{-1}$ (Figure 1b). A similar relationship was observed between the diltiazem-induced hypotension and tachycardia (Figure 3). This probably reflects a greater cardiac slowing effect of *trans*-diclofurime and diltiazem in the presence of reflex tachycardia. Thus *trans*-diclofurime and diltiazem behaved like typical group II antagonists in the present study, whereas verapamil caused a significantly greater degree of tachycardia for a given fall in MAP and the effects were not significantly different from those of nifedipine in this respect.

The observations of the present study in conscious SHR concerning the different degrees of tachycardia induced by diltiazem, verapamil and nifedipine are consistent with the findings of previous studies. It has been shown that i.v. administered verapamil and nifedipine frequently cause tachycardia in man (Lydtin *et al.*, 1975; Vincenzi *et al.*, 1976; Rowland *et al.*, 1979; Mitchell *et al.*, 1982) and in conscious dogs (Gross *et al.*, 1979; Walsh *et al.*, 1981; Nakaya *et al.*,

1983), whereas diltiazem produces little increase in heart rate in the clinic (Oyama 1979; Mitchell *et al.*, 1982) or in conscious dogs (Walsh *et al.*, 1981; Nakaya *et al.*, 1983). Considering that verapamil is equipotent with *trans*-diclofurime in inhibiting contractility in guinea-pig atria (Spedding *et al.*, 1987), slowing of atrioventricular conduction in pithed rats (Spedding, 1982; Spedding *et al.*, 1987) and is also more or equipotent with diltiazem for the negative chronotropic effect on the sinus node preparation, inhibition of atrioventricular conduction (Taira, 1979) and for direct cardiac effects in pithed rats (Spedding, 1982), it was rather unexpected to find that verapamil caused significantly greater tachycardia than *trans*-diclofurime and diltiazem in the present study. The precise reason(s) for this anomaly is not clear but it is becoming increasingly apparent that the calcium antagonists, apart from inhibiting calcium influx at the level of the slow calcium channel in blood vessels and heart, also have additional sites of action which may not be primarily related to their calcium channel blocking properties. In this respect verapamil has been shown to have interactions at various other neurotransmitter receptors and processes (see for review De Feudis, 1987).

It has been found that at least two binding sites exist for group II calcium antagonists in the calcium channel with different selectivities for the phenylalkylamines, such as verapamil, and the benzothiazepines, like diltiazem (Glossman *et al.*, 1982; Glossman & Ferry, 1983; Boles *et al.*, 1984; Schoemaker *et al.*, 1987). The present findings, that in conscious SHR the cardiovascular effects of *trans*-diclofurime closely resemble those of diltiazem but not those of nifedipine or verapamil are consistent with the suggested molecular mode of action of *trans*-diclofurine at the diltiazem binding site in the calcium channel (Mir & Spedding, 1987).

In conclusion, *trans*-diclofurime is a potent antihypertensive agent in the conscious SHR, the hypotensive effects being associated with less reflex tachycardia than is normally associated with calcium antagonists such as nifedipine and verapamil.

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