Effects of Mebudipine and Dibudipine, Two New Calcium-channel Blockers, on Rat Left Atrium, Rat Blood Pressure and Human Internal Mammary Artery

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Abstract

Mebudipine and dibudipine are two new dihydropyridine calcium-channel blockers that have been synthesized in our laboratory. In a previous study, they showed considerable relaxant effect on vascular and ileal smooth muscle. Here, the pharmacological effects of mebudipine and dibudipine on isolated rat left atrium, rat blood pressure and isolated human internal mammary artery are described. Results are compared with those obtained for nifedipine.

Mebudipine and dibudipine reduced contraction force of rat left atrium (pIC30 values: $5 \cdot 37 \pm 0 \cdot 13$ and $5 \cdot 49 \pm 0 \cdot 15$, respectively) but their negative inotropic effects were significantly weaker than that of nifedipine (pIC30 value: $6 \cdot 63 \pm 0 \cdot 11$). Mebudipine and dibudipine lowered rat blood pressure. The hypotensive effect of mebudipine was similar to that of nifedipine while dibudipine was weaker than nifedipine. It was found that the half-life of the hypotensive action of dibudipine ($41 \cdot 91 \pm 3 \cdot 77$ min, $31 \cdot 13 \pm 2 \cdot 26$ min and $28 \cdot 20 \pm 4 \cdot 37$ min at 2, 4 and $8 \, \text{mg kg}^{-1}$ orally administered doses, respectively) was longer than that of nifedipine ($11 \cdot 85 \pm 2 \cdot 88$ min, $16 \cdot 65 \pm 2 \cdot 42$ min and $14 \cdot 03 \pm 0 \cdot 10$ min at the same doses, respectively). Also, it appeared that mebudipine had a slower rate of absorption compared with nifedipine (the time to reach peak hypotensive action at 2, 4 and $8 \, \text{mg kg}^{-1}$ orally administered doses were, respectively, $24 \cdot 00 \pm 6 \cdot 96$ min, $23 \cdot 75 \pm 2 \cdot 39$ min and $15 \cdot 00 \pm 2 \cdot 04$ min for mebudipine and $7 \cdot 80 \pm 0 \cdot 86$ min, $13 \cdot 75 \pm 3 \cdot 15$ min and $8 \cdot 33 \pm 0 \cdot 88$ min for nifedipine). The two new compounds, as well as nifedipine, relaxed KCl-treated isolated human internal mammary artery (pEC50 values; $7 \cdot 87 \pm 0 \cdot 12$, $7 \cdot 22 \pm 0 \cdot 24$ and $7 \cdot 67 \pm 0 \cdot 12$ for mebudipine, dibudipine and nifedipine, respectively). The relaxant effects of mebudipine and dibudipine did not show any significant difference compared with that of nifedipine.

It is concluded that these new compounds are weak cardiodepressants and, with due attention to its significant vasorelaxant action, mebudipine is a vasoselective compound. In addition, these two compounds have potent blood pressure lowering effects. Also, their vasorelaxant action can be reproduced in human vascular preparations.

Calcium-channel blockers have a significant role in the treatment of several cardiovascular and non-cardiovascular disorders (Weiner 1988; Parmley 1996). Currently, extensive research is being carried out on the synthesis of new compounds of this class. Synthesis of compounds with greater tissue selectivity, longer duration of action and slower rate of absorption is the main aim of the current efforts. Such improvements in the properties of new drugs will ultimately lead to fewer side effects and improved patient compliance. In our previous study (Mahmoudian et al 1997) it was shown that mebudipine and dibudipine, two new 1,4-dihydropyridine derivatives synthesized in our laboratory, were potent relaxants of vascular and ileal smooth muscle and that their potencies in these

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preparations were comparable with that of nifedipine. Also it appeared that mebudipine had a higher affinity toward depolarized tissue compared with nifedipine.

In the present study the pharmacological effect of these two new compounds on isolated rat left atrium, rat blood pressure and isolated human internal mammary artery has been studied. Our aim was to compare the potencies of these compounds in other preparations and more importantly, compare their pharmacological profile with that of a standard 1,4-dihydropyridine derivative (i.e. nifedipine).

Materials and Methods

Drugs and solutions
Nifedipine was a gift from Tolid-darou Pharmaceuticals. For in-vitro studies, all three calcium-channel blockers were dissolved in dimethyl sulphoxide (DMSO) to produce 1 mM solutions. These were then further diluted with distilled water. For in-vivo studies, polyethylene glycol 400 (PEG 400) was used as a solvent. Solutions of dihydropyridines and the organ bath were protected from light.

Isolated rat left atrium
Male rats (local breed of Sprague–Dawley origin), 220–250 g, were killed by a blow on the head and decapitation. The heart was excised and the left atrium was dissected free and suspended in an organ bath filled with physiologic salt solution of the following composition (in mM): NaCl 119, KCl 4·7, CaCl$_2$ 2·5, KH$_2$PO$_4$ 1·2, MgSO$_4$ 1·2, NaHCO$_3$ 25, glucose 11. The physiologic salt solution was continuously gassed with a mixture of 95% O$_2$ and 5% CO$_2$ and its temperature was maintained at 32°C. The fluid of the organ bath was changed every 10 min. The resting tension of the left atrium was adjusted to 0·5 g and the tissue allowed to equilibrate for 1 h.

In order to induce contractions in the left atrium, the tissue was stimulated with a Harvard stimulator (frequency = 1 Hz, pulse width = 5 ms, voltage = 20% above threshold) using a bipolar platinum electrode. Contractions of the left atrium were recorded using a force transducer coupled to a Beckman physiograph (model R511A). To study the cardiodepressant action of mebudipine, dibudipine and nifedipine, increasing concentrations of each compound were added to the organ bath and changes in contraction force were recorded. The

pIC30 (−log IC30) value for each compound was calculated from concentration–response curves.

Rat blood pressure
Normotensive white male rats (local breed of Sprague–Dawley origin), weighing 200–300 g, were anaesthetized with 2·5–3 mL kg^{-1} of 25% aqueous urethane solution, intraperitoneally. Suitable depth of anaesthesia was maintained by injecting small doses of urethane solution. The unconscious animal was placed on an electrical blanket thermostated at 37°C.

Measurement of systolic blood pressure was performed using the tail-cuff method. This method was used in order to evaluate the survival rate of animals after drug therapy. The pulses in the tail artery were recorded by means of a pneumatic sensor and a pneumatic pulse transducer on a Narco physiograph (model MK-III-S). To study the effect of drugs on rat blood pressure, single doses of each drug were administered both intraperitoneally (2 mg kg^{-1}) and orally (2, 4 and 8 mg kg^{-1}). The volume of administered drug was 2 mL kg^{-1}.

Isolated human internal mammary artery
Human internal mammary artery segments were collected from 22 patients undergoing coronary artery bypass surgery. Use of discarded internal mammary artery tissue was approved by the ethical committee of the Heart Hospital. During the operation, the discarded distal end of the internal mammary artery was carefully removed and placed in ice-cold physiological solution. The time-delay from the operation room to laboratory was about 20 min. At the laboratory, the internal mammary artery was carefully dissected from surrounding tissues and then was cut into 4–5 rings of 3 mm length. The rings were joined together using surgical thread and suspended in organ bath with one end connected to a hook and the other end to a force transducer. The resting tension was adjusted to 4 g and the tissue was allowed to equilibrate for 90 min. The physiologic salt solution composition was as follows (in mM): NaCl 118, KCl 4·8, CaCl$_2$ 2·5, KH$_2$PO$_4$ 1·2, MgSO$_4$ 1·2, NaHCO$_3$ 25, glucose 10. The solution was bubbled with a mixture of 95% O$_2$ and 5% CO$_2$ at 37°C. The fluid of the organ bath was changed every 15 min.

In order to study the effect of mebudipine, dibudipine and nifedipine on human internal mammary artery, the tissues were pre-contracted with K$^+$ (40 mM) after which cumulative concentrations of the compounds were added into the organ bath. The concentration of each compound

which produced half-maximal relaxation (EC50) was used as an indicator of the potency of the compound.

Statistical methods

Results are expressed as means ± s. e. Results were analysed using the two-tailed Student's t-test. A *P* value <0·05 was considered to be significant. The half life of the hypotensive effect of each drug was calculated using Drug Kinetic software (Mirfazaelian & Mahmoudian 1995).

Results

Effect on the isolated rat left atrium

Mebudipine, dibudipine and nifedipine dose-dependently reduced contraction force of the rat left atrium (Figure 1). The pIC30 values of mebudipine and dibudipine were smaller than that of nifedipine (Table 1). In other words, the negative inotropic effect of mebudipine and dibudipine was significantly smaller than that of nifedipine (*P* < 0·001 for mebudipine vs nifedipine, *P* < 0·005 for dibudipine vs nifedipine).

Effect on rat blood pressure

Intraperitoneal administration of mebudipine, dibudipine and nifedipine resulted in a long-lasting hypotensive effect (Figure 2). Mebudipine and nifedipine did not show any significant difference with each other in their hypotensive effect but both

Table 1. The potencies of mebudipine, dibudipine and nifedipine for reduction of contraction force of isolated rat left atrium and relaxing precontracted isolated rat aorta.

	Mebudipine	Dibudipine	Nifedipine
pIC30 (Atrium)	5·37 ± 0·13	5·49 ± 0·15	6·36 ± 0·11
pIC50 (Aorta)[a]	8·61 ± 0·09	7·59 ± 0·12	8·29 ± 0·07
IC30 (Atrium)/ IC50 (Aorta)	1738	126	85

Values are means ± s.e. The negative inotropic effect of mebudipine and dibudipine are significantly lower than that of nifedipine (mebudipine compared with nifedipine, *P* < 0·001; dibudipine compared with nifedipine *P* < 0·005). [a]pIC50 values for relaxing precontracted isolated rat aorta are taken from a previous study (Mahmoudian et al 1997).

of them were significantly more potent than dibudipine in this respect (*P* < 0·025 after reaching maximum effect).

Oral administration of a single dose of mebudipine, dibudipine and nifedipine (2, 4 and 8 mg kg^{-1}) resulted in a transient hypotensive effect (Figure 3). The maximum hypotensive effects of oral administration of mebudipine and nifedipine in all three doses were greater than that of dibudipine (*P* < 0·0025) but the two former drugs did not differ significantly from each other in this respect (Table 2). No animal death was observed following the highest oral dose (8 mg kg^{-1}) of mebudipine or dibudipine. Thus it can be concluded that these two new compounds, at least at the above mentioned dose, do not cause acute toxicity. The half-life of the hypotensive action of dibudipine, at all three administered
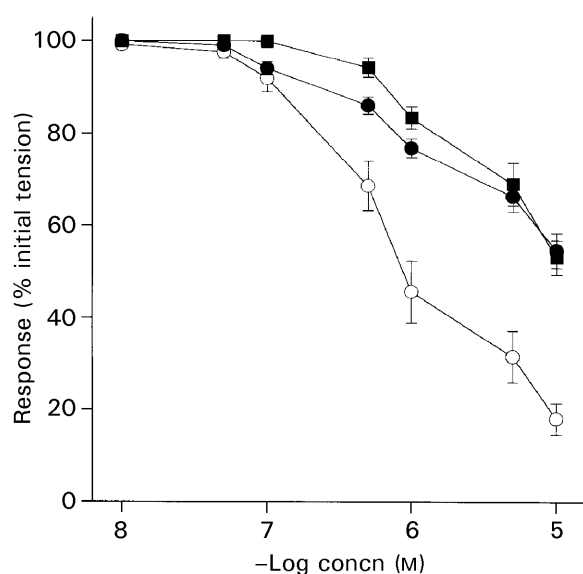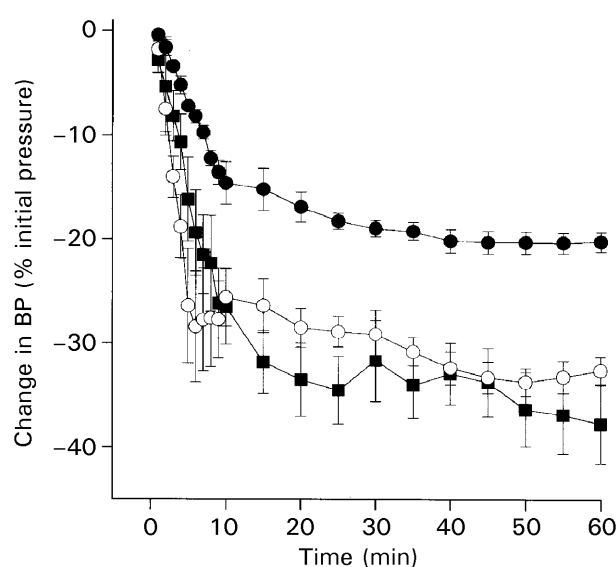

Figure 1. The inhibitory effect of increasing concentrations of mebudipine (■), dibudipine (●) and nifedipine (○) on contraction force of isolated rat left atrium. Results are means ± s.e.m., n = 5.

Figure 2. The blood pressure lowering effect of intraperitoneal administration of 2 mg kg^{-1} mebudipine (■), dibudipine (●) and nifedipine (○) in unconscious rats. Results are means ± s.e.m., n = 6.
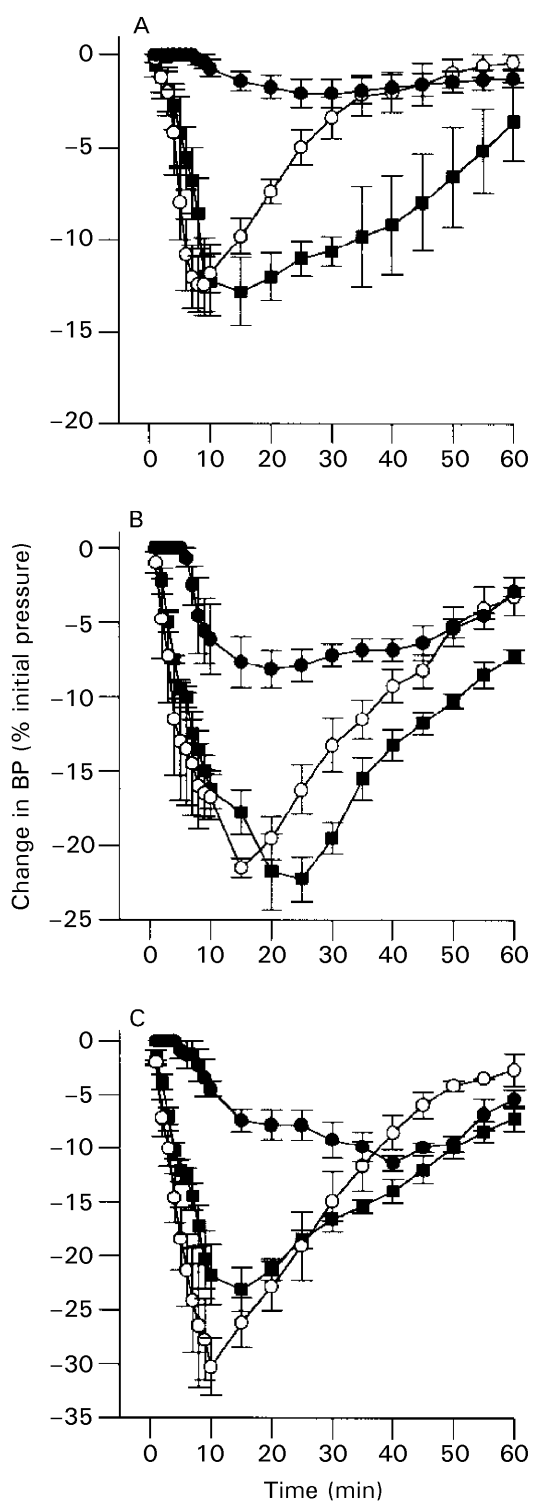
Figure 3. The blood pressure lowering effect of oral administration of $2\,mg\,kg^{-1}$ (A), $4\,mg\,kg^{-1}$ (B) and $8\,mg\,kg^{-1}$ (C) mebudipine (■), dibudipine (●) and nifedipine (○) in unconscious rats. Results are means ± s.e.m., n = 4–5.

Table 2. The maximal hypotensive effect (percentage of the maximum reduction of the basal blood pressure) of mebudipine, dibudipine and nifedipine after administration of a single oral dose to rats.

Dose ($mg\,kg^{-1}$)	Δ BP (% initial pressure)		
	Mebudipine	Dibudipine	Nifedipine
2	12·80 ± 1·85	2·10 ± 0·78	12·40 ± 1·54
4	22·25 ± 1·49	8·13 ± 1·27	21·50 ± 0·65
8	23·08 ± 2·04	11·30 ± 0·72	30·72 ± 2·71

Results are means ± s.e. from 4–5 rats. Mebudipine and nifedipine, at all 3 doses used, did not differ significantly from each other in the maximum obtainable hypotensive effect, but both were more potent than dibudipine in this respect ($P < 0.0025$).

Table 3. The half-lives of hypotensive action of mebudipine, dibudipine and nifedipine after administration of a single oral dose to rats.

Dose ($mg\,kg^{-1}$)	Half-life (min)		
	Mebudipine	Dibudipine	Nifedipine
2	22·16 ± 7·61	41·91 ± 3·77	11·85 ± 2·88
4	21·39 ± 1·17	31·13 ± 2·26	16·65 ± 2·42
8	27·52 ± 3·95	28·20 ± 4·37	14·03 ± 0·10

At all 3 doses used, the half-life of hypotensive action of dibudipine was significantly longer than that of nifedipine (at $2\,mg\,kg^{-1}$ $P < 0.001$, at $4\,mg\,kg^{-1}$ $P < 0.01$, at $8\,mg\,kg^{-1}$ $P < 0.05$). Only at $8\,mg\,kg^{-1}$ did mebudipine show a significant hypotension half-life longer than nifedipine

dose (Table 3). The time to reach peak hypotensive effect of each compound is shown in Table 4. At all three doses used, the time to peak hypotensive effect of mebudipine was longer than with nifedipine ($P < 0.05$). Since the maximum hypotensive effect of these two drugs were similar (Table 2), it seems that the rate of absorption of mebudipine is slower than for nifedipine.

Effect on isolated human internal mammary artery
Mebudipine, dibudipine and nifedipine relaxed isolated human internal mammary artery rings precontracted by KCl (40 mM) (Figure 4). Since the maximum relaxation induced by each compound differed in each experiment, the EC50 value (concentration which produced half maximal relaxation) of each compound was used to compare their potencies. It was found that the potency of mebudipine and dibudipine in the relaxation of K^+-induced contraction of internal mammary artery did not differ significantly from that of nifedipine, while the potency of mebudipine in eliciting this effect was greater than that of dibudipine ($P < 0.05$, Table 5).

doses, was significantly longer than that of nifedipine (Table 3). Comparing mebudipine and nifedipine, the former had a significantly longer hypotensive action half-life only at the $8\,mg\,kg^{-1}$

Table 4. Time to reach peak hypotensive effects of mebudipine, dibudipine and nifedipine after administration of a single oral dose to rats.

Dose (mg kg^{-1})	Time to peak effect (min)		
	Mebudipine	Dibudipine	Nifedipine
2	24.00 ± 6.96	26.67 ± 1.67	7.80 ± 0.86
4	23.75 ± 2.39	14.33 ± 3.48	13.75 ± 3.15
8	15.00 ± 2.04	36.67 ± 3.33	8.33 ± 0.88

At all 3 doses used, the time to reach maximal hypotensive effect was longer for mebudipine compared with nifedipine ($P < 0.05$).
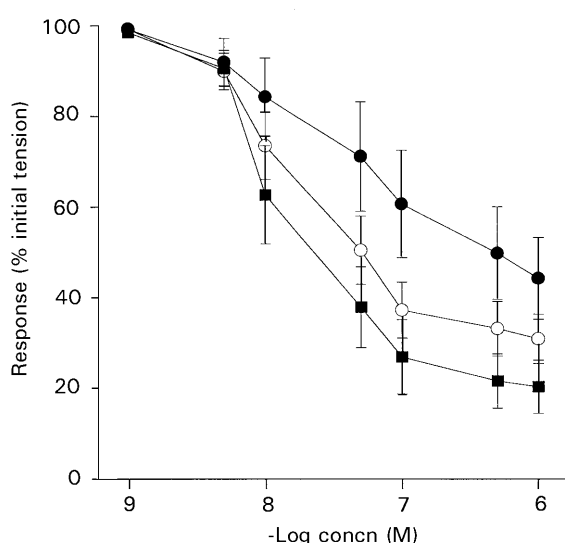

Figure 4. The relaxing effect of increasing concentrations of mebudipine (■), dibudipine (●) and nifedipine (○) on isolated human internal mammary artery precontracted by KCl (40 mM). Results are means ± s.e.m., n = 7–8.

Discussion

In our previous study, mebudipine and dibudipine showed potent vasodilatory effects (Mahmoudian et al 1997). In the present study, these two compounds reduced the contraction force of rat left atrium. This effect is due to a reduction of calcium entry through the plateau phase of the atrial action potential. The negative inotropic effects of these compounds are significantly lower than that of nifedipine (Table 1). From division of the calculated IC30 values of these compounds in rat left atrium by their IC50 values for relaxing rat aorta contraction (Mahmoudian et al 1997), it appears that mebudipine is a more vasoselective compound than nifedipine. This special characteristic of mebudipine, makes it a suitable calcium-channel

Table 5. pEC50 ($-\log$ EC50) values for relaxation by mebudipine, dibudipine and nifedipine of isolated human internal mammary artery, precontracted by KCl.

	Mebudipine	Dibudipine	Nifedipine
pEC50	7.87 ± 0.12	7.22 ± 0.24	7.67 ± 0.12

Values are means ± s.e. The differences between the potencies of mebudipine and nifedipine, and between dibudipine and nifedipine, were not statistically significant but mebudipine was more potent than dibudipine ($P < 0.05$).

blocker for administration to patients with heart failure. It is worthwhile noting that among the present classes of calcium-channel blockers, dihydropyridine derivatives have the least cardiodepressant action, but even when using nifedipine life-threatening deterioration of cardiac action has been reported in patients with severe heart failure (Elkayam et al 1985; Pieper 1996). It appears that in such patients, mebudipine might be a safer drug than nifedipine. Mebudipine showed a greater relaxant effect toward depolarised tissue in guinea-pig ileal smooth muscle (Mahmoudian et al 1997). Taking into consideration the fact that vascular smooth muscle has a higher resting membrane potential compared with cardiac tissue, the greater affinity for the depolarized state might explain the vasoselectivity of mebudipine. It must be mentioned that while all of the dihydropyridine derivatives have greater affinity for calcium channels in depolarized tissue, the magnitude of this voltage-dependent binding varies (Ferrari et al 1994; Godfraind 1994).

It has been shown that vasoselective calcium-channel blockers have a cardioprotective action in ischaemia-induced cardiac injury (Ehring et al 1992). This is due to the fact that the resting membrane potential of ischaemic areas is higher than that of non-ischaemic areas. Selective binding to depolarized ischaemic tissues reduces calcium overload in these areas and decreases cardiac cell injury (Ehring et al 1992; Heusch 1992). It is possible that mebudipine, with its marked vasoselective action, could be an ischaemic-selective and cardioprotective compound. Further studies have to be performed to confirm this speculation.

In anaesthetized rats, the blood pressure lowering effect of mebudipine and nifedipine are similar after both intraperitoneal and oral administration and these two compounds are significantly more potent than dibudipine (Figures 2 and 3). An interesting observation when dibudipine is administered orally is its long-lasting hypotensive effect. In the three doses used in this study, the half-life of the hypotensive action of dibudipine is longer than

that of nifedipine (Table 3). If this property is confirmed in future pharmacokinetic studies, it would indicate that dibudipine might have a special advantage in many disorders (e.g. hypertension, angina pectoris) in which calcium-channel blockers are used for long periods, since a long duration of action reduces administration times and improves patient compliance (Salvetti & Di Venanzio 1994). In addition, it has been observed that 24-h control of hypertension and reduction of blood pressure fluctuation using long-acting antihypertensive drugs, provides a better prognosis for hypertensive patients (Borchard 1994; Salvetti & Di Venanzio 1994).

The longer time taken by mebudipine to reach peak hypotensive effect compared with nifedipine (Table 4), is another important finding of the present study. The slower rate of absorption of mebudipine is important to note since a slow rise in plasma concentration of a calcium-channel blocker reduces the occurrence of some side effects (e.g. reflex tachycardia, flushing, headache, dizziness) (Borchard 1994). In addition, since it is claimed that using short-acting formulations of nifedipine increases mortality (Furberg et al 1995) and that probably the most important reason for this effect is drug-induced reflex tachycardia (Furberg et al 1995; Kloner 1995; Opie & Messerli 1995), using compounds with a slow rate of absorption would be safer. The slow rate of absorption of mebudipine needs to be confirmed in further pharmacokinetic studies.

It should be noted that no apparent trend was observed in the time taken to reach peak effect on the blood pressure as doses were increased. This may be due to the discrepancy between pharmacodynamic and pharmacokinetic time-courses of these drugs' actions at different dose levels.

The two newly synthesized compounds, as well as nifedipine, relax isolated human internal mammary artery pre-contracted with KCl (Figure 4). Their EC50 values to elicit this effect did not significantly differ from that of nifedipine (Table 5). The relaxing effect of mebudipine and dibudipine in human internal mammary artery confirms their vasorelaxant effect on human arteries. In addition, it points to their effectiveness in the control of vasospasm of human internal mammary artery which occasionally occurs during coronary artery bypass surgery (He et al 1989).

In conclusion, in comparison with nifedipine, mebudipine and dibudipine have weak cardiodepressant action and mebudipine shows a significant vasoselectivity. The hypotensive effect of mebudipine is comparable with that of nifedipine while dibudipine is weaker than nifedipine in this respect. It seems that the hypotensive action of dibudipine lasts longer than that of nifedipine. Mebudipine is absorbed more slowly than nifedipine. Mebudipine and dibudipine show a vasorelaxant effect on human as well as animal artery preparations.

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