J. Pharm. Pharmacol. 1990, 42: 325–331
Received February 27, 1990

Effects of Verapamil, Nifedipine and Flunarizine on Haemodynamics and Regional Blood Flows in Pentobarbitone-anaesthetized Rats

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Abstract—Representative calcium antagonists from proposed class I (nifedipine), class II (verapamil) and class III (flunarizine) have been examined for effects on blood pressure, heart rate, ventricular pressures, ECG, cardiac output and regional blood flow in pentobarbitone-anaesthetized rats. Flow was measured by the microsphere technique. Low and high infusion rates of each drug were chosen to decrease mean arterial pressure by 25 and 40 mmHg, respectively. At equi-depressor infusion rates, all drugs equally decreased total peripheral resistance and slightly increased cardiac output and stroke volume. Heart rate was decreased by verapamil and flunarizine, but increased by nifedipine. Verapamil markedly decreased dP/dt_{max} of ventricular pressure and prolonged the PR-interval. Flunarizine was similar. Nifedipine decreased dP/dt_{max} and had no effect on the PR-interval of the ECG. Similar effects on regional blood flow were seen with the three drugs; flow to lungs, heart, liver, skeletal muscle and stomach increased. Correction for blood pressure changes, i.e. conductance, showed that vasodilatation occurred in all regions, with all drugs, except for the skin. Therefore, representatives from three sub-classes of calcium antagonists had similar effects on blood flow but different effects on the heart.

Calcium antagonists were originally classified into two subgroups by Fleckenstein (1983). Group A consisted of the more potent and specific drugs, and included verapamil, nifedipine and diltiazem, while group B included drugs such as prenylamine, fendiline and caroverine. Spedding (1985) classified calcium antagonists on the basis of lipophilicity, chemical structure and pharmacological activity. Group 1 included the 1,4-dihydropyridines such as nifedipine and nimodipine, while group 2 included structurally-diverse agents such as verapamil and diltiazem, which are basic compounds with similar lipophilicities. Group 3 contained diphenylalkylamines such as cinnarizine and flunarizine. In-vitro experiments indicate that the three classes bind to different sites on the calcium channel and display different pharmacological actions (Spedding 1984; Spedding & Berg 1984).

Several studies have determined the effects of various calcium antagonists on systemic haemodynamics and blood flow distribution in conscious rats (Flaim & Zelis 1982; Kanda & Flaim 1984), anaesthetized rats (Gulati et al 1983) and open-chest anaesthetized cats (Hof et al 1982; Hof 1983). No study has yet compared the cardiovascular effects of representative drugs from the three subclasses in the same preparation, using a common solvent for the drugs. Normally, nifedipine is compared with its solvent, ethanol, and other drugs with their respective solvents. A fair comparison of different calcium antagonists requires the use of a common solvent for all drugs. The purpose of the present study was, therefore, to investigate whether representative drugs from Spedding's (1985) subclasses have similar profiles of cardiovascular actions under conditions of a common solvent. The effects of low and high infusion rates of nifedipine (class I), verapamil (class II) and flunarizine (class III) on heart rate, left ventricular pressure and dP/dt_{max}, cardiac output, ECG and blood flow distribution were investigated in pentobarbitone-anaesthetized rats. The low and high infusion rates of each drug were chosen to decrease mean arterial pressure by 25 and 40 mmHg, respectively, in the presence of a common solvent.

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Materials and Methods

Preparation of rats

Male Sprague-Dawley rats (Charles River, Canada) were anaesthetized with pentobarbitone (60 mg kg^{-1}). The right iliac artery was cannulated for measurement of mean arterial blood pressure and the right femoral vein for administration of drug. A left iliac arterial cannula was used to remove blood samples. Cannulae (PE 50) were inserted into the left ventricle via the right carotid artery for injection of microspheres and measurement of left ventricular pressure and dP/dt_{max} by electronic differentiation. Heart rate was determined electronically from the arterial pulse pressure. ECG recordings were taken from Lead I and PR and QRS intervals were measured (Budden et al 1980).

Experimental protocol

Experiments were performed according to a blind and random design after preliminary experiments had established suitable low and high infusion rates of each drug to cause 25 and 40 mmHg falls in mean blood pressure, respectively. Drug doses were: low rate verapamil (V_L), 43 $\mu g\ kg^{-1}\ min^{-1}$; high rate verapamil (V_H), 83 $\mu g\ kg^{-1}\ min^{-1}$; low rate nifedipine (N_L), 12 $\mu g\ kg^{-1}\ min^{-1}$; high rate nifedipine (N_H), 36 $\mu g\ kg^{-1}\ min^{-1}$; low rate flunarizine (F_L), 174 $\mu g\ kg^{-1}\ min^{-1}$, and high rate flunarizine (F_H), 275 $\mu g\ kg^{-1}\ min^{-1}$. The

common solvent was 0·014% tartaric acid in 30% ethanol in 0·9% NaCl (saline). At the infusion rate used, a total dose of 0·2 g kg^{-1} ethanol was given. Stock solutions (verapamil, 1 mg mL^{-1}; nifedipine, 0·3 mg mL^{-1}; flunarizine, 2 mg mL^{-1}) were prepared and protected from light at all times and infusions were made using opaque syringes and cannulae. After surgery, rats were left for 30 min before beginning the experiment by injecting the first set of microspheres (pre-drug). Drug or vehicle was then infused at 0·07 mL min^{-1} for 12 min before the second set of microspheres (post-drug) was injected.

Microspheres

Cardiac output and blood flow distribution were determined by the microsphere technique using 15 μm diameter microspheres, labelled with either ^{57}Co or ^{113}Sn (New England Nuclear), as described elsewhere (Pang 1983). To avoid variation in ^{57}Co and ^{113}Sn microsphere distribution, their order of injection was reversed in half of the experiments.

When the estimates of blood flow in left and right kidneys in any rat differed by more than 20%, inadequate ventricular mixing of microspheres was assumed to have occurred and the animal was rejected from the study. Whole organs, except for 30 g representative samples of muscle and skin from areas with unimpaired vasculature, were excised and gamma emission counted by a Searle 1185 series dual channel automatic gamma counter with a 3 inch NaI crystal at energy settings of 80–160 keV and 330–480 keV for ^{57}Co and ^{113}Sn, respectively. At these energy settings, the "spill-over" from ^{57}Co into ^{113}Sn was negligible (0·03%). Spillover of ^{113}Sn into the ^{57}Co channel was 16% and a correction was made for this.

Data and statistical analyses

Total peripheral resistance, cardiac output and blood flows were calculated as previously described (Pang 1983). Conductance was calculated by dividing blood flow by mean arterial pressure, and stroke volume by dividing cardiac output by heart rate. ANOVA (repeated measures) was used to compare all data obtained during the first and second injections of the microspheres. To ensure normality of data for blood flow and conductance, changes were logarithmically-transformed before analyses. Duncan's multiple range test (Montgomery 1984) was used to compare group means. A probability of error, $P < 0·05$, was preselected as the level of statistical significance. Results are presented as mean ± s.e.m.

Results

Haemodynamics, heart rate and ECG

Before treatment with drug, or solvent alone, there were no significant differences between the various groups with regard to mean arterial blood pressure, cardiac output, left ventricular pressure, heart rate, stroke volume or the PR and QRS intervals of the ECG. The range of pretreatment values and overall means are shown in Table 1.

The purpose of the study was to compare the haemodynamic effects of equi-depressor doses of representative drugs from the three subclasses of calcium antagonists. The representative drugs verapamil, nifedipine, and flunarizine, at the low doses, lowered mean arterial pressure by 31, 28 and 30 mmHg, respectively, from 113 ± 6 to 82 ± 2, 118 ± 5 to 90 ± 5 and 124 ± 6 to 94 ± 4 mmHg. At the high doses, they

Table 1. Range of pretreatment values for all rat groups.

	A. Haemodynamic and ECG variables	
Haemodynamics/ECG	Range of Group means	Overall mean ± s.e.m.
Mean arterial pressure (MAP, mmHg)	110–124	117 ± 13
Cardiac output (CO, mL)	77–102	87 ± 34
Total peripheral resistance (TPR, mmHg min mL^{-1})	1·3–1·5	1·4 ± 0·2
Left ventricular pressure (LVP, mmHg)	138–154	147 ± 15
dP/dt_{max} (mm Hg s^{-1})	7560–8530	8121 ± 1164
Heart rate (HR, beats min^{-1})	344–401	372 ± 44
Stroke volume (SV, mL)	0·22–0·27	0·23 ± 0·05
PR interval (PRL, ms)	46–51	48 ± 4·5
QRS interval (ms)	34–38	35 ± 2·6

	B. Pretreatment blood flow (mL min^{-1})		C. Pretreatment conductance (mL min^{-1} $mmHg^{-1}$)	
Tissue	Range of group means	Overall mean ± s.e.m.	Range of group means	Overall mean ± s.e.m.
Lungs	1·1–2·1	1·4 ± 0·9	0·9–1·9	1·3 ± 0·8
Heart	4·0–5·2	4·6 ± 1·3	3·4–4·8	4·0 ± 1
Liver	1·7–2·7	2·1 ± 1·1	1·5–2·4	1·9 ± 0·9
Stomach	1·0–1·5	1·2 ± 0·5	0·8–1·2	1·0 ± 0·8
Intestine	10·1–13·8	11·5 ± 3·4	9·2–11·6	10·0 ± 2·8
Caecum/Colon	3·9–5·1	4·3 ± 1·6	3·4–4·2	3·7 ± 1·2
Kidneys	15·3–20·9	17·5 ± 4·4	13·6–17·4	15·2 ± 3·4
Spleen	1·1–1·9	1·5 ± 0·7	0·9–1·7	1·3 ± 0·6
Muscle	1·5–1·9	1·7 ± 0·6	1·3–1·8	1·5 ± 0·6
Skin	2·2–3·3	2·5 ± 1·3	1·9–2·7	2·2 ± 0·9
Testes	1·3–1·6	1·4 ± 0·3	1·0–1·3	1·2 ± 0·3
Brain	1·3–1·7	1·5 ± 0·5	1·1–1·6	1·3 ± 0·5

lowered mean arterial pressure by 41, 42 and 38 mmHg, respectively, from 110±3 to 69±3, 121±4 to 79±4 and 114±2 to 76±4 mmHg. The fact that the effects of the doses of the drugs we chose may have been threshold in some blood vessels, and maximal in others, should show which vascular beds were more susceptible to the vasodilating effects of the different calcium antagonists and allow comparison between representative members of the three subclasses of such drugs.

The solvent was not entirely without actions since it reduced mean arterial blood pressure by 15% as a result of intestinal vasodilatation; there was a small, but statistically insignificant, fall in total peripheral resistance and a statistically significant increase in intestinal flow and conductance. There were no statistically significant changes in cardiac output or in flow in other vascular beds. The fall in blood pressure was, however, associated with a significant fall in left ventricular developed pressure.

The effects of treatment on the different variables are summarised in Fig. 1. All treatments produced significant falls in blood pressure; at the higher doses, the falls were twice those produced by solvent alone (not shown). As expected, these findings reflected the doses which had been chosen in trial experiments to produce comparable blood pressure falls. In all cases the changes in blood pressure were accompanied by statistically significant falls in total peripheral resistance. Associated with this was a tendency for cardiac output to rise but such rises were not consistently significant with all doses except with flunarizine.

All treatments reduced both ventricular pressure and dP/dt_{max}, the largest reductions appearing with verapamil which, at the lower dose, was also more effective than flunarizine in reducing heart rate. Nifedipine, unlike verapamil and flunarizine, elevated heart rate. Apart from an increase after high dose verapamil, there were no significant drug effects on the P-R interval. Thus, verapamil had the strongest cardiodepressant effect and nifedipine had none.

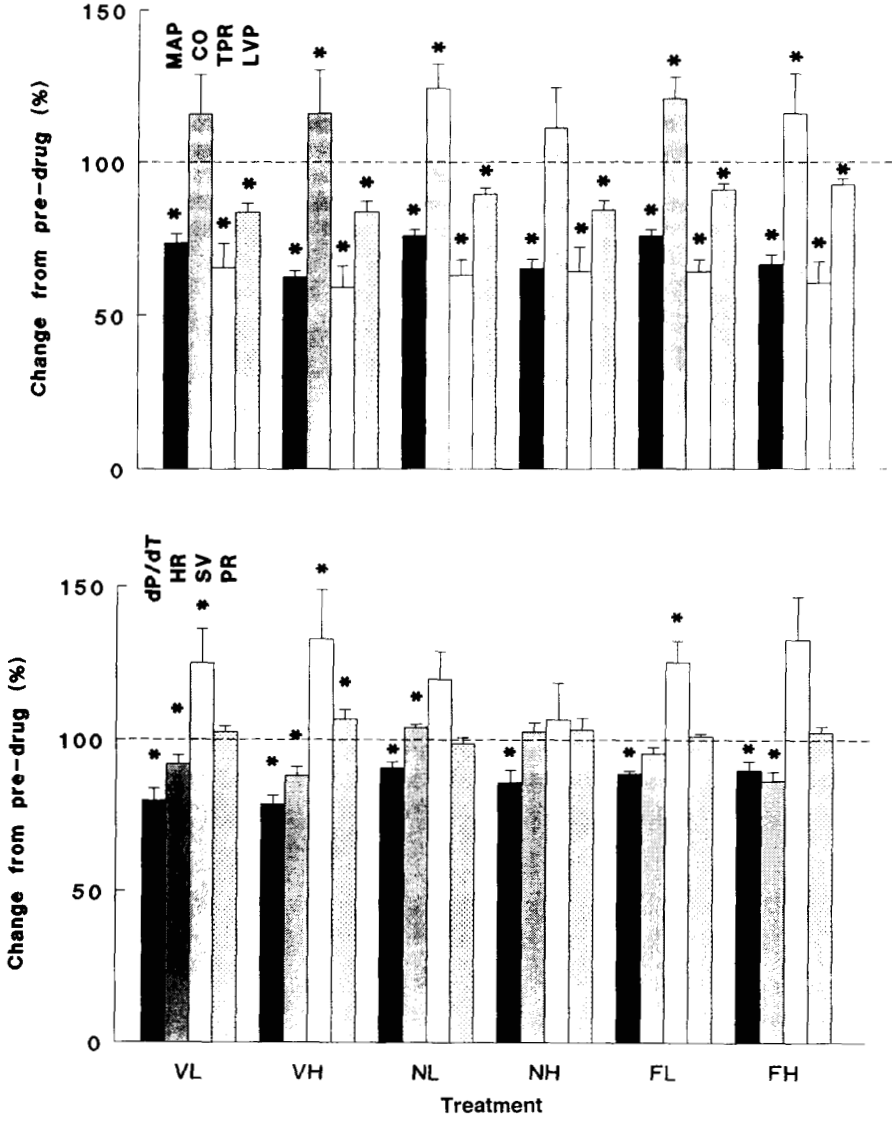

FIG. 1. Effects of a low dose (L) and a high dose (H) of verapamil (V), nifedipine (N) and flunarizine (F) on haemodynamics and ECG in pentobarbitone-anaesthetized rats. Each column represents mean±s.e.m.; n=8. See Table 1A for abbreviations and units. * Denotes statistical significance from pretreatment values ($P<0.05$).

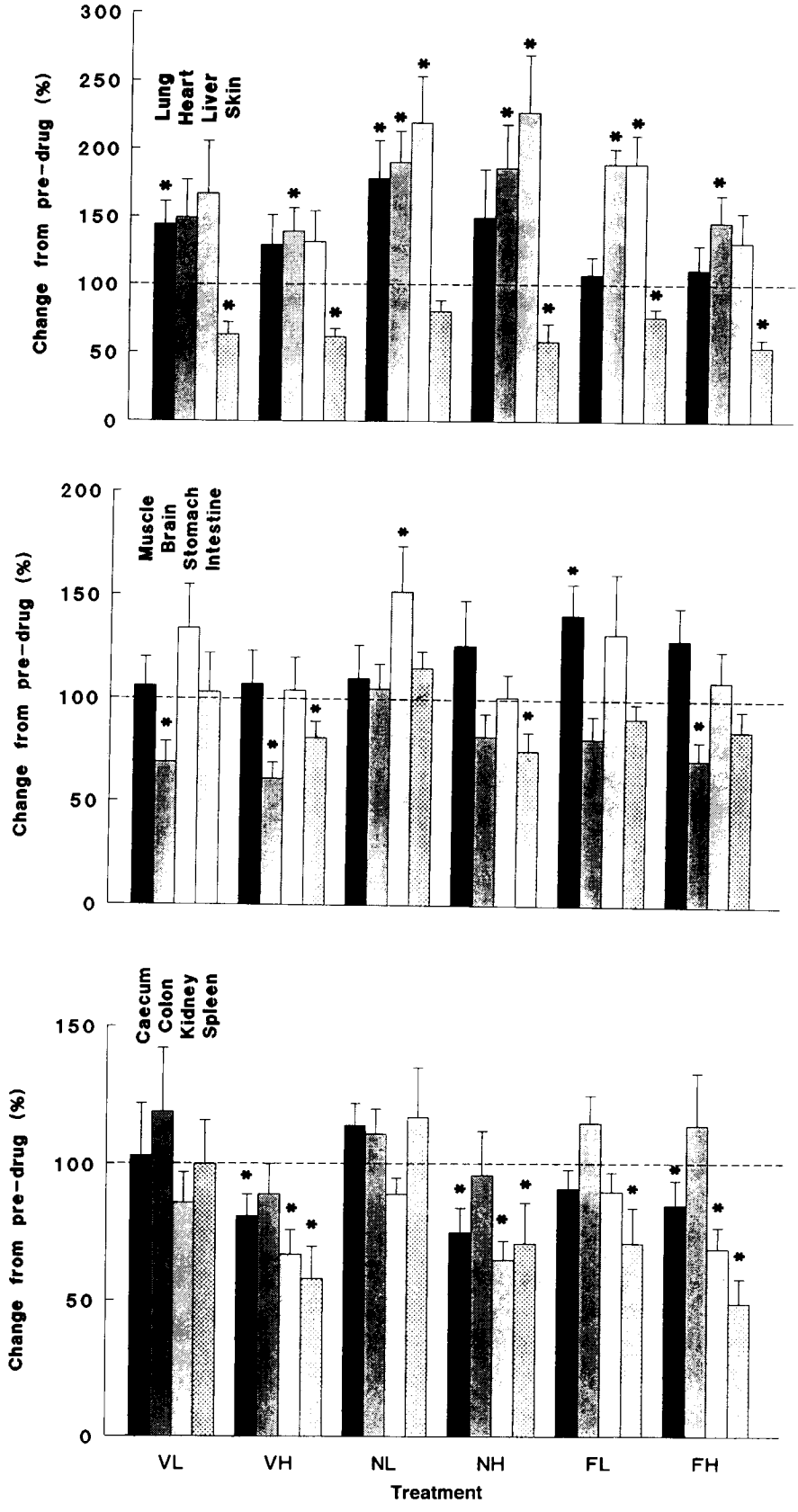

FIG. 2. Effects of a low dose and a high dose of verapamil, nifedipine and flunarizine on organ blood flow in pentobarbitone-anaesthetized rats, abbreviations as in Fig. 1. Each column represents blood flow (mean ± s.e.m.; n = 8) to whole organs, except for skin (30 g) and muscle (30 g). * Denotes drug effects which are statistically significant from pretreatment values ($P < 0.05$).

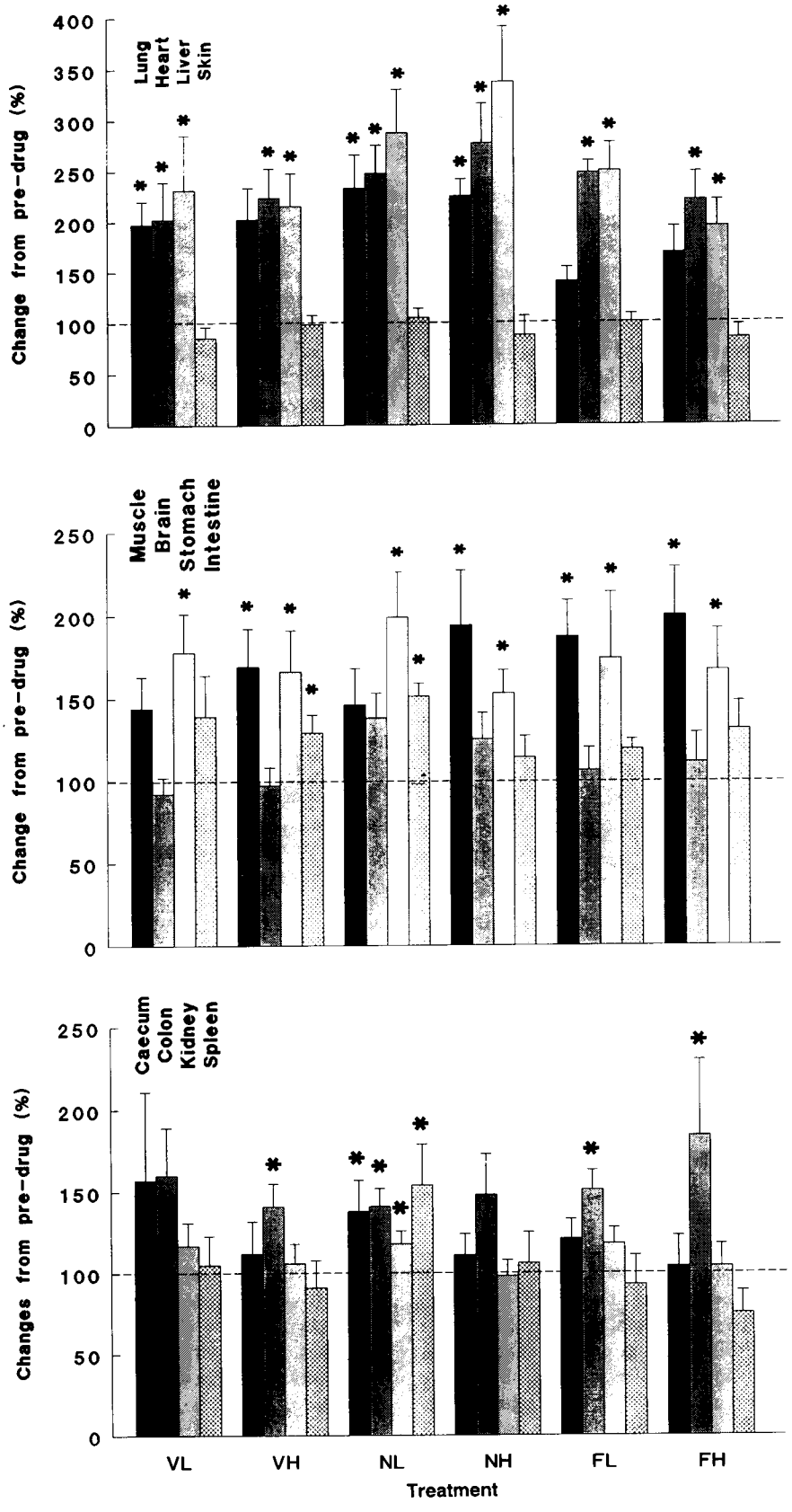

FIG. 3. Effects of a low dose and a high dose of verapamil, nifedipine and flunarizine on arterial conductance in pentobarbitone-anaesthetized rats, abbreviations as in Fig. 1. Each column represents conductance (mean ± s.e.m.; n = 8) to whole organs, except for skin (30 g) and muscle (30 g). * Denotes drug effects which are statistically significant from pretreatment values ($P < 0.05$).

Effects on regional blood flows and conductances

Before treatment, no statistically significant variation in regional flow or conductance was found between the different groups of animals. The ranges of group means of pre-drug flow and conductance values, together with the pooled means, are shown in Table 1B, C.

There was an increase in mean blood flow with all drug treatments in the lungs, heart, liver, muscle and stomach. These increases reached statistical significance in the lungs for low doses of verapamil and nifedipine, in the heart for all the drugs except the low dose verapamil, in the liver for nifedipine and low dose flunarizine, in the muscle for low dose flunarizine and in the stomach for low dose nifedipine (Fig. 2). Nifedipine appeared to increase flow in these organs more than verapamil or flunarizine, but apart from blood flow in the liver ($P<0{\cdot}05$) such differences were not significant. There was a tendency for decreased flow in other beds; this reached statistical significance in the kidneys, spleen, intestine, caecum, skin and brain. The vehicle alone, in contrast to the calcium antagonists, caused a significant increase in intestinal flow, but did not significantly alter flow in other vascular beds.

However, changes in flow can give a misleading view of vasodilatation, so flows were normalized for changes in blood pressure (i.e., conductance was calculated) to prevent this. Following this normalization, it can be seen from Fig. 3 that there was a pattern of vasodilatation with increased conductance in most vascular beds, except for the skin where there were no changes of conductance. The increases in conductance reached statistical significance for all drugs in heart, liver, muscle, stomach and colon. As was the case with flow, nifedipine appeared to be more efficacious than verapamil or flunarizine, e.g., in the heart and liver, but these inter-drug differences in conductances were not statistically significant.

Discussion

In this study, the profile of haemodynamic actions and blood flow effects was similar for the three calcium antagonists with nifedipine being the most potent, on a weight basis, and flunarizine the least. At equi-depressor doses, all three drugs were similar in their actions on cardiac output, total peripheral resistance and blood flow. On the other hand, the drugs were different with respect to effects on the heart. Thus, flunarizine at high dose and verapamil both slowed heart rate. Nifedipine caused a small increase in heart rate, perhaps due to reflex action. There were no effects on the PR interval, except for a small but significant increase with high dose verapamil. Cardiac contractility, as measured by dP/dt_{max}, was decreased by nifedipine and flunarizine, but even more so by verapamil. These differences in action between verapamil, flunarizine and nifedipine may reflect a relative selectivity of the drugs for cardiac versus vasculature tissue. Thus, if both high and low dose responses are considered, these representatives of the three subclasses of calcium antagonists proposed by Spedding (1985) can apparently be differentiated according to their effects on the heart.

In spite of a decrease in cardiac contractility, as measured by dP/dt_{max}, cardiac output and stroke volume were not decreased but increased by the drugs, often significantly. In conscious rats, there was a similar dissociation from cardiac contractility; cardiac output and stroke volume were not altered significantly by nifedipine (Kanda & Flaim 1984) or diltiazem (Flaim & Zelis 1982). There was a tendency for cardiac output to be increased in chloralose-anaesthetized open-chest cats by PY 108–068, nicardipine, verapamil, diltiazem (Hof 1983) and nifedipine (Hof et al 1982). The ability of all the calcium antagonists to maintain cardiac output, in spite of a decrease of myocardial contractility, may be due to a decrease in arteriolar tone resulting in a decrease in flow resistance and elevated venous tone via reflex mechanisms. We have shown that total body venous tone is increased by verapamil (Waite et al 1988), however, after ganglionic blockade with hexamethonium, verapamil decreased venous tone.

In contrast to their effects on the heart, the three drugs had qualitatively similar effects on tissue blood flow and flow conductances. However, in those beds in which flow and conductance was increased by the greatest amount (heart and liver), nifedipine was more efficacious than verapamil and flunarizine, and flunarizine was the most effective in increasing flow and conductance in muscle. While the effects on blood flow indicate the major effects were increased flow in the lungs, heart, liver, skeletal muscle and stomach, normalization for falls in blood pressure (i.e. conductance estimation) showed that vasodilatation occurred in most beds with the exception of the skin.

Calcium antagonists have been reported to increase coronary (Flaim & Zelis 1982; Hof et al 1982; Hof 1983; Kanda & Flaim 1984) and hepatic arterial flow (Kanda & Flaim 1984). The increase in hepatic flow may have been a regulatory phenomenon secondary to a decrease of portal venous flow; it has been shown that a decrease in portal venous flow generally leads to an increase in hepatic arterial flow such that total hepatic blood flow remains constant (Lautt 1980). The reduction of blood flow to the kidneys and brain seen in the present experiments may have been due to disturbance of autoregulation. Calcium antagonists either have no effect or decrease renal blood flow (Flaim & Zelis 1982; Hof et al 1982; Gulati et al 1983; Hof 1983; Kanda & Flaim 1984) while at the same time decreasing flow to the skin and spleen (Kanda & Flaim 1984).

Conductance reflects active changes in vascular tone more accurately than does blood flow. The decrease seen in the skin was probably the result of release of vasoconstrictor agents following falls in blood pressure. Decrease in blood pressure results in reflex activation of vasopressor systems, including the sympathetic nervous, renin-angiotensin, and vasopressin systems. Infusions of verapamil in conscious sheep (Mzail & Noble 1986) and nifedipine in anaesthetized dogs (Imagawa et al 1986) have been shown to increase plasma renin activity. In anaesthetized rats, endogenously-released vasopressin was a prominent vasoconstrictor in skin and stomach, while angiotensin II caused the greatest vasoconstriction in skin and kidneys (Pang 1983). The sympathetic nervous system, on the other hand, had the greatest vasoconstrictor influence in lungs and skeletal muscle (Tabrizchi & Pang 1987). Thus, blood flow redistribution seen with the calcium antagonists appears to be a consequence of direct vasodilatation and reflex vasoconstriction.

In summary, nifedipine was overall the most efficacious drug in changing flow and conductance, especially in the liver, and the least efficacious one in myocardial depressant effects. Verapamil was clearly the most efficacious with respect to cardiac depressant effects. Flunarizine was generally less efficacious despite causing the same fall in blood pressure as the other two drugs. Apart from its ability to increase flow and conductance in muscle, and its failure to affect lung blood flow, flunarizine had no unusual vascular effect. It also lay mid-way between nifedipine and verapamil in its cardiac depressant effects. Our results provide some justification of attempts to classify the calcium antagonists on the basis of differential effects on cardiac and vascular tissue.

Acknowledgements

This work was supported by the British Columbia Heart Foundation. We would like to thank Knoll Ag., Ludwigshaven, Germany for the supply of verapamil HCl and Bayer, Leverkusen, Germany for the supply of nifedipine.

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