Comparison of Vasodilator Effects of DN-9693, a Selective Inhibitor of Cyclic AMP Phosphodiesterase, and Isobutylmethylxanthine, a Non-Selective One, in Dogs

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Abstract—The present experiments were performed in anesthetized dogs in order to determine if DN-9693, a new antiplatelet agent known to selectively inhibit cyclic AMP phosphodiesterase (PDE), and isobutylmethylxanthine (IBMX), which inhibits both cyclic AMP and GMP PDEs, have different cardiovascular actions. With intra-arterial administration into the left anterior descending coronary, femoral, cranial mesenteric and renal arterial beds perfused at constant pressure with autologous blood, both agents increased blood flow in a similar dose range. DN-9693 was longer-acting than IBMX. Both agents were nearly equi-effective in the femoral circulation, but DN-9693 was 1.5–2 times less effective than IBMX in the others. With intravenous administration, both agents were equi-effective in increasing the maximum rate of rise of left ventricular pressure, heart rate and myocardial oxygen consumption and in reducing mean blood pressure. However, DN-9693 was less effective in increasing coronary sinus outflow than IBMX. These results suggest the following: 1) Vasodilation in the somatic rather than the visceral circulation is important in reducing mean blood pressure. 2) Cyclic GMP may not be involved in the cardiac action of PDE-inhibitors. 3) Cyclic GMP may be involved in the vasodilator effect of PDE-inhibitors in the coronary, mesenteric and renal circulations but least involved in the femoral circulation.

It is well-known that inhibitors of cyclic nucleotide phosphodiesterase (PDE) produce vasodilatation. However, there have been no systematic studies to determine if the vasodilator actions of these agents are qualitatively similar in different arterial beds and differ only in potency. This question has arisen in the minds of the present authors because nearly all PDE inhibitors have more or less additional actions (1–5), and because recently three isozymes of cyclic nucleotide PDEs have been identified and some PDE inhibitors have been shown to inhibit more selectively one of the three isozymes than do others (6). DN-9693, 7-pyperidinyl-1,2,3,5-tetrahydroimidazo [2,1-b]-quinazolin-2-one HCl, which has been recently developed as an antiplatelet drug is one of the former type of PDE inhibitors. DN-9693 has been shown to inhibit selectively cyclic AMP PDE (isozyme III) in platelets (7). In contrast, 3-isobutyl-1-methylxanthine (IBMX) which has been used as a standard agent to inhibit cyclic AMP PDE because of lack of additional action (8) has inhibitory effects on both cyclic AMP and cyclic GMP PDEs (9). The present experiments were designed to determine if these two PDE inhibitors having different inhibitory spectra have differential vasodilator profiles in four arterial beds, i.e., the coronary, femoral, mesenteric and renal arterial beds of dogs. Because coronary blood flow is influenced by the metabolism of the myocardium, the effects of the two agents on myocardial
oxygen consumption (\( \text{MVO}_2 \)) were also investigated.

Materials and Methods

Experiments were carried out exclusively on mongrel dogs of either sex anesthetized with sodium pentobarbital (30 mg/kg, i.v.).

Coronary and peripheral circulations: Dogs weighing 8–16 kg were used. Only in the experiments on coronary circulation, dogs were respired artificially with room air in a tidal volume of 20 ml/kg at 18 breaths/min by means of a dog respirator (Harvard Apparatus, model 607), because the experiments were carried out under conditions in which the chest was opened in the 5th intercostal space on the left side. Otherwise, dogs were allowed to respire spontaneously. After injection of 500 U/kg, i.v., of calcium heparin, the left anterior descending coronary (LAD), the right femoral, the cranial mesenteric or the right renal artery was cannulated, and their vascular beds were perfused with autologous blood from the left common carotid artery. Calcium heparin was added at a dose of 100 U/kg, i.v., at hourly intervals. Arterial blood was delivered by means of a peristaltic pump (Harvard Apparatus, model 1215). Constant pressure perfusion was achieved by the use of a Starling pneumatic resistor through which a fraction of the blood was shunted to the left jugular vein. Perfusion pressure was kept constant at a value slightly higher than the mean blood pressure. Blood flow in each arterial bed was measured by an electromagnetic flowmeter (Nihon Kohden, MFV-2100). Systemic blood pressure was measured from the left femoral artery with a pressure transducer (Nihon Kohden, MPV-0.5) and heart rate determined with a cardiotachometer (San-ei Instrument, Type 2140) triggered by blood pressure pulses. Left ventricular pressure (LVP) was measured with a high-fidelity micromanometer-tipped angiocatheter (Miller Instruments, Mikro-tip PC-360) or a pressure transducer (Nihon Kohden, TP-200T) via a Mallinckrodt angiocatheter introduced into the left ventricle through the left carotid artery. LV dP/dt max was obtained, and instantaneous and mean systemic blood pressure was measured as described in the preceding section. For i.v. administration of agents, the left femoral vein was cannulated, and drug solutions were injected in a volume of less than 1.5 ml and flushed in with 2 ml of Tyrode’s solution.

Drugs: The drugs tested were DN-9693
Differential Effects of DN-9693 and IBMX (Aldrich Chemical). Both agents were dissolved in and diluted with 0.9% saline to the desired concentrations.

**Statistical analysis:** All changes in vascular and cardiac variables except for arteriovenous O_2 difference (A-V O_2 difference) produced by each dose of both agents were expressed as percentage changes from their predrug values. Changes in A-V O_2 difference were expressed as percentage changes from the initial value before administration of 0.03 μmol/kg of each agent. The reason for this was as follows: frequent blood sampling tended to deteriorate the condition of the animals.

Experimental values were presented as the mean±S.E., unless otherwise stated. The difference in paired or unpaired mean values was analyzed by the t-test and judged to be significant when P values were less than 0.05. Differences between dose-response curves were analyzed by analysis of variance of two-way layout. The slopes of dose-response curves were referred to those of their regression lines, and parallelism of dose-response curves was analyzed by the covariance techniques described by Snedecor and Cochran (11).

**Results**

**Effects on coronary and peripheral circulations**

The initial values of mean blood pressure and heart rate in the 24 dogs used in this series of experiments were 110±4 mmHg and 165±5 beats/min, respectively. The initial values of coronary (LAD), femoral, mesenteric and renal blood flow were 13.4±0.7 (n=6), 53.5±6.6 (n=6), 87.5±6.1 (n=6) and 69.2±1.7 (n=6) ml/min, respectively. When perfusion pressure (mean±S.D.) was 110±18, 132±13, 121±9 and 138±10 mmHg, respectively.

I.a. injections of DN-9693 (1–1,000 nmol) and IBMX (1–1,000 nmol) produced a dose-related increase in blood flow (vasodilation) in all four arterial beds.

**Coronary circulation:** One of the experiments with DN-9693 and IBMX is shown in Fig. 1, and dose-vasodilator response curves to both agents are presented in Fig. 2A. Analysis of variance showed that there was a significant difference between these two dose-response curves (P<0.01). The slope of the dose-response curve for DN-9693 was such that coronary blood flow increased by about 10% with a 10-fold increase in doses and was about 2 times less steep than that for IBMX (Table 1). As seen in Fig. 1, the vasodilator responses to higher doses of DN-9693 were longer-lasting than those of IBMX. The half-durations of vasodilator responses, periods from the point of the half peak response to that of the half recovery, to 3, 10, 300 and 1000 nmol of DN-9693 were significantly (P<0.01) longer than those of the corresponding doses of IBMX. Both agents increased LV dP/dt max in a dose-related manner (Fig. 2B). Their dose-response curves were nearly identical.

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**Fig. 1.** Effects of i.a. DN-9693 and IBMX on blood flow through the left anterior descending coronary artery in an anesthetized dog.
and, LV dP/dt max increased by about 15% with a 10-fold increase in doses of both agents.

**Femoral circulation:** The effects of the two agents on femoral blood flow are shown in Fig. 3. There was no significant difference between their dose-vasodilator response curves. Their slopes were such that blood flow increased by about 25% with a 10-fold increase in doses (Table 1). There was no significant difference in half-duration of vasodilator response between corresponding doses of the two agents.

**Mesenteric circulation:** Figure 4 shows the effects of the two agents on mesenteric blood flow. There was a significant difference between the dose-response curves to these agents (P<0.01); although vasodilator responses to both agents were almost identical in their peak values at lower doses, the responses to IBMX became greater than those of DN-9693 at higher doses. The slope of the curve for DN-9693 was such that a 10-fold increase in doses resulted in about a 30% increase in blood flow, and the slope of the curve for IBMX was about 1.4 times steeper than that for DN-9693 (Table 1). The half-durations of the vasodilator responses to both agents were nearly equal at all corresponding doses.

**Renal circulation:** Figures 5 and 6 show the effects of the two agents on renal blood flow. As clearly seen in these figures, their vasodilator effects were weak. Nevertheless,
there was a significant difference between the two dose-response curves (P<0.01). The slope of the curve for DN-9693 was such that only about a 4.5% increase in blood flow resulted from a 10-fold increase in doses (Table 1). The slope of the curve for IBMX was about 1.6 times steeper than that for DN-9693 (Table 1). The half-durations of the vasodilator responses to both agents were nearly equal at doses below 30 nmol, but those of DN-9693 became longer at doses above 100 nmol and significant differences were observed at 100 (P<0.01) and 1000 nmol (P<0.05).

**Effects on myocardial O₂ consumption (MVO₂) and systemic circulation**

The initial cardiohemodynamic values in two groups of dogs, each consisting of 6 animals, one for DN-9693 and the other for IBMX were respectively as follows: Mean blood pressure, 90±10 and 86±9 mmHg; coronary sinus outflow, 30±3 and 28±3 ml/min; coronary arterial resistance, 3.2±0.5 and 3.2±0.2 mmHg/ml; A-V O₂ difference, 9.7±2.4 and 14.2±1.7 vol. %; MVO₂, 4.9±1.1 and 7.1±1.2 ml/min per 100 g; heart rate, 134±6 and 167±11 beats/min; LV dP/dt max 1997±203 and 2650±582 mmHg/sec.

The dose-response curves for cardiohemodynamic effects of DN-9693 and IBMX are shown in Fig. 7. In doses of 0.03–1 μmol/kg, both DN-9693 and IBMX increased coronary sinus outflow, heart rate and LV dP/dt max and decreased coronary resistance and mean blood pressure in a dose-related manner. However, IBMX was more effective in increasing coronary sinus outflow (P<0.01). A 10-fold increase in doses resulted in about 15% increase in coronary sinus outflow with DN-9693 and about 45% increase with IBMX (Table 2). The decrease in coronary resistance was about 23% with DN-9693 and about 33% with IBMX (Table 2). Duration of coronary vasodilator action of
DN-9693 was longer than that of IBMX (data not shown). There were no significant differences in dose-response curves between DN-9693 and IBMX for other cardiohemodynamic variables. Namely, mean blood pressure decreased by about 15%, heart rate by about 10% and LV dP/dt max increased by about 20% with a 10-fold increase in doses of both agents (Table 2). Coronary A-V O₂ difference was not markedly changed by both agents, although it tended to increase with DN-9693 and tended to decrease with IBMX at higher doses. There was no significant difference between the dose-response curves for change in MVO₂ to both agents. It increased by about 25% with a 10-fold increase in doses of both agents (Table 2).

**Fig. 6.** Dose-response curves to i.a. DN-9693 (open circles) and IBMX (solid circles) for increase in blood flow through the right renal artery. Data points are means±S.E. (n=6).

**Fig. 7.** Dose-response curves to i.v. DN-9693 (open circles) and IBMX (solid circles) on coronary sinus outflow, mean blood pressure, coronary resistance, heart rate, A-V O₂ difference, MVO₂ and LV dP/dt max. Data points are means±S.E. (n=6). *P<0.05 and **P<0.01 when compared with the respective initial values.
Table 2. The slopes of the dose-response curves to DN-9693 and IBMX on coronary sinus outflow (CoF), mean blood pressure (MBP), coronary arterial resistance (CoR), heart rate (HR), arteriovenous $O_2$ difference (A-V $O_2$), myocardial $O_2$ consumption (MVO$_2$) and left ventricular (LV) $dP/dt$ max.

<table>
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<th>Agent</th>
<th>CoF</th>
<th>MBP</th>
<th>CoR</th>
<th>HR</th>
<th>A-V $O_2$</th>
<th>MVO$_2$</th>
<th>LV $dP/dt$ max</th>
</tr>
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<td>15.4</td>
<td>-15.3</td>
<td>-23.0</td>
<td>11.7</td>
<td>17.7</td>
<td>27.3</td>
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</tr>
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<td>-16.1</td>
<td>-33.0</td>
<td>10.3</td>
<td>-6.2</td>
<td>25.2</td>
<td>19.3</td>
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**Discussion**

In the present experiments DN-9693 and IBMX, when administered i.a., increased blood flow in the coronary, femoral, mesenteric and renal arterial beds in a similar dose range. Since in the present experiments these arterial beds were perfused at constant pressure, increases in blood flow reflect directly decreases in arterial resistance. Thus, the present results can be described in such a way that both agents decreased arterial resistance in the four arterial beds in a similar dose range. However, the vasodilator profiles of both agents were not identical in all the four arterial beds. In the femoral arterial bed, both DN-9693 and IBMX showed essentially identical dose-response curves with a slope of about a 25% increase in blood flow with a 10-fold increase in doses. However, in the other arterial beds, IBMX was more effective in increasing blood flow than DN-9693: in the coronary arterial bed, IBMX was about 2 times as effective as DN-9693; and in the mesenteric and renal arterial beds, IBMX was about 1.5 times as effective as DN-9693. When administered i.v., both agents reduced mean blood pressure and coronary arterial resistance, and they increased coronary sinus outflow in a similar dose range. In increasing coronary sinus outflow, IBMX was about 3 times as effective as DN-9693; and in decreasing coronary resistance, IBMX was about 1.5 times more effective than DN-9693. Although the value concerning the relative effectiveness of IBMX to DN-9693 is slightly different depending upon whether changes in coronary sinus outflow or those in coronary resistance were taken for comparison, IBMX being more effective than DN-9693 in coronary circulation held with both i.a. and i.v. administration.

With i.v. administration, both DN-9693 and IBMX were nearly equi-effective in reducing mean systemic blood pressure; a 10-fold increase in doses resulted in about a 15% decrease in mean systemic blood pressure. With i.a. administration, both agents were nearly equi-effective only in the femoral arterial bed, although in this arterial bed, a 10-fold increase in doses resulted in about a 25% increase in blood flow. It is of interest that the effectiveness of the two agents in reducing mean blood pressure is more closely related to the effectiveness in producing femoral arterial dilatation than that in producing vasodilatation of the mesenteric and renal arterial beds. This suggests that dilatation of the somatic arterial bed plays a more important role than that of the visceral arterial bed in reducing mean blood pressure with vasodilators with PDE-inhibitory action.

With i.a. administration into the LAD and also with i.v. administration of the two agents, LV $dP/dt$ max increased in a similar way, although i.v. administration was slightly more effective than i.a. administration. With i.v. administration, MVO$_2$ increased with both agents in a similar way. Heart rate was also increased in a similar way by both agents. Thus, the cardiac effects of PDE inhibitors appear to be independent of whether they inhibit selectively cyclic AMP PDE or inhibit non-selectively both cyclic AMP and GMP PDEs. This is conceivable since available information indicates that cyclic GMP plays no significant role in any of the functions of cardiac cells (12-14).

In spite of the equi-effectiveness of both agents on cardiac variables (including MVO$_2$) as described above, IBMX was more effective...
than DN-9693 in producing coronary vasodilatation. A similar relation held also in the mesenteric and renal arterial beds. Thus, it is tempting to speculate that in these arterial beds, an increase in cyclic GMP resulting from non-selective inhibition of PDEs would also contribute to the vasodilator effect of PDE-inhibitors, and this mechanism would make IBMX more effective than DN-9693 which selectively inhibits cyclic AMP PDE. Furthermore, the greater effectiveness of IBMX in the coronary circulation than in other circulations appears to reflect the greater involvement of cyclic GMP in the vasodilator effect of PDE inhibitors. However, the following questions should be answered before such conclusions are drawn: 1) Are there differences in vasodilator activity between cyclic AMP and cyclic GMP in these arterial beds? 2) Are there differences in distribution or activity of cyclic AMP and cyclic GMP PDEs in these arterial beds? 3) How much are mechanisms of action other than PDE inhibition involved in the vasodilator effect of these agents?

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References