In Vivo Analysis of Adrenergic Receptor Activity of Dobutamine

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ABSTRACT

Studies were performed in anesthetized dogs to evaluate the cardiac and systemic effects of intravenously administered dobutamine and to determine its direct effects on the renal and femoral vascular beds. The results demonstrated that dobutamine possessed an inotropic efficacy similar to that of isoproterenol and norepinephrine; its chronotropic effect was similar to or greater than that of norepinephrine. In contrast to norepinephrine, dobutamine increased cardiac output and reduced total peripheral resistance with minimal effects on mean aortic pressure. Studies on the denervated hind limb demonstrated that dobutamine stimulated both alpha and beta receptors. The dose of dobutamine which produced a 50% increase in femoral blood flow was 180 times the required dose of isoproterenol and the dose which produced a 50% increase in contractile force was 43 times the required dose of isoproterenol. Studies on the renal vasculature demonstrated that dobutamine caused no dopamine-like renal vasodilator activity and only minor vasodilation mediated by beta receptors. We concluded that dobutamine is more cardioselective than is isoproterenol. The dobutamine-induced decrease in peripheral resistance observed in the whole dog was presumably due to increased myocardial contractility coupled with a greater net effect of beta-adrenergic vasodilation than alpha-adrenergic vasoconstriction. Studies with reserpine-treated dogs showed that all dobutamine-induced effects were due to a direct action on receptors.

KEY WORDS

cardioselectivity  cardiac beta receptors  peripheral beta receptors  norepinephrine  isoproterenol  dopamine  imipramine

Dobutamine is a novel sympathomimetic amine currently being evaluated as a treatment for cardiac insufficiency (1, 2). The drug can produce a significant increase in myocardial contractility and cardiac output with minor effects on blood pressure (1-3).

Considered as a group, catecholamines cause a spectrum of blood pressure changes ranging from pressor to depressor effects. Several catecholamines, including epinephrine (4) and dopamine (5), have little effect on blood pressure at doses producing a significant increase in myocardial contractile force. The overall hemodynamic effect of epinephrine, dopamine, and dobutamine is an increase in cardiac output with a reciprocal decrease in total peripheral resistance. Although both epinephrine and dopamine are alpha-receptor agonists, infusion of moderate doses produces vasodilation due to a preponderance of peripheral beta-receptor activity (6) or dopaminergic activity (7), respectively. In the case of dobutamine, the basis of the decreased total peripheral resistance has not been established. Although McRitchie et al. (3) have suggested that the beta-receptor activity of the compound is cardioselective, preliminary results from our laboratory (8) have indicated both direct alpha- and beta-receptor effects on the vasculature of the canine hind limb at doses that produce a positive inotropic effect.

The present studies were undertaken to compare the cardiac effects of dobutamine, norepinephrine, and isoproterenol in relation to their effects on hind-limb flow, to determine whether dobutamine possesses selective renal vasodilator activity similar to that of dopamine, and to compare the overall hemodynamic effects of dobutamine and norepinephrine in intact dogs anesthetized with chloralose.

Methods

SYSTEMIC HEMODYNAMICS

Thirteen mongrel dogs of either sex were anesthetized with chloralose (90 mg/kg, iv) and placed on positive-pressure respiration with room air. An external jugular vein and a femoral artery were cannulated for the infusion of drugs and the measurement of arterial blood pressure with a Statham P23D pressure transducer. The chest was then opened through a left

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lateral thoracotomy. Left ventricular pressure was monitored with a high-fidelity pressure transducer surgically implanted in the apex of the left ventricle. The first derivative of ventricular pressure (dP/dt) was determined with an active operational amplifier circuit. Myocardial contractility was determined from the ratio of the rate of rise of pressure to the instantaneous common peak isovolumic pressure; this method has previously been shown to minimize the effects of preload and afterload (9). Cardiac output was monitored with an electromagnetic flow probe placed around the ascending aorta. The flow probe was calibrated in situ post-mortem. All parameters, including the electrocardiogram, were recorded on an Electronics-for-Medicine DR12 recorder. Total peripheral resistance was calculated by dividing mean arterial blood pressure by cardiac output.

Dobutamine1 was infused over a range of doses from 2 μg/kg min⁻¹ to 32 μg/kg min⁻¹; hemodynamic measurements were performed 5 minutes after parameters had stabilized at each dose. In six additional dogs dose-response curves to continuously infused norepinephrine (0.125–0.5 μg/kg min⁻¹) were determined. Doses were varied in step increments.

CARDIAC FUNCTION

Eight mongrel dogs of either sex were anesthetized with sodium pentobarbital (30 mg/kg, iv) followed by sodium barbital (150 mg/kg, ip). The vagus were sectioned bilaterally, and respiration was maintained with a Harvard pump. Mean arterial blood pressure was monitored via a catheter inserted in a brachial artery. A jugular vein was cannulated for intravenous injections of drugs. A right thoracotomy was performed, and the left kidney was exposed through a flank incision, and the renal artery was dissected free of surrounding tissue. Renal blood flow was determined with a Statham electromagnetic flowmeter. Drugs were injected in 0.2 ml of 0.95% saline via a 23-gauge needle inserted into the renal artery proximal to the flow probe. Patency of the needle was maintained by a slow infusion of 0.95% saline.

We used a previously established method to screen for dopaminergic renal vasodilation (11, 12). Renal blood flow responses to isoproterenol (0.16–40.5 nmoles), dopamine (0.62–633.5 nmoles), and dobutamine (2.76–2,825 nmoles) were investigated after infusion of phenoxybenzamine (5 mg/kg, ia). Following these determinations, propranolol (1 mg/kg, ia) was infused, and the drug sequence was repeated.

MECHANISM OF ACTION

Four dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv). Mean arterial blood pressure was monitored via a catheter inserted in a femoral artery connected to a Statham P23D pressure transducer. The left kidney was exposed through a flank incision, and the renal artery was dissected free of surrounding tissue. Renal blood flow was determined with a Statham electromagnetic flowmeter. Drugs were injected in 0.2 ml of 0.95% saline via a 23-gauge needle inserted into the renal artery proximal to the flow probe. Patency of the needle was maintained by a slow infusion of 0.95% saline.

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Following these studies, the same dogs were studied for peripheral alpha-receptor activity as described in a preceding section. Propranolol (1 mg/kg, ia) was administered and dose-response relationships were determined for norepinephrine and dobutamine. Subsequently, the effect of imipramine (5 mg/kg, iv) on the dose-response curves of norepinephrine and dobutamine was studied.

Results

SYSTEMIC HEMODYNAMICS

The hemodynamic effects of dobutamine and norepinephrine in dogs with intact baroreceptor

1Kindly supplied by Dr. Ronald R. Tuttle of the Lilly Research Laboratories.
reflexes are illustrated in Figure 1. In contrast to the slight reflex slowing of heart rate by norepinephrine, dobutamine produced a positive chronotropic response at higher doses. Dobutamine had little effect on mean arterial blood pressure, but it increased cardiac output and reduced total peripheral resistance. Norepinephrine, however, did not increase cardiac output but increased mean arterial blood pressure and total peripheral resistance. Both drugs produced dose-related increases in contractility.

**CARDIAC FUNCTION**

Contractile force dose-response curves for isoproterenol, norepinephrine, and dobutamine were essentially parallel in dogs with and without reserpine treatment. Reserpine produced no significant effect on the myocardial response to the three amines (Fig. 2). The relative doses required to double contractile force were isoproterenol 1, norepinephrine 3, and dobutamine 43. Figure 3 illustrates that all three amines produced positive chronotropic effects and that treatment with reserpine did not attenuate these effects. Figure 4 illustrates that, for an equivalent inotropic effect, isoproterenol exerted a greater chronotropic effect than did norepinephrine or dobutamine and that there was no apparent difference between the effects of norepinephrine and dobutamine.

**HIND-LIMB HEMODYNAMICS**

Pilot studies demonstrated that the response to intra-arterial injections of dobutamine was variable with a slight vasoconstriction occurring at low doses and a biphasic response occurring at higher doses. Since phenoxybenzamine blocked the vasoconstrictor component and propranolol blocked the vasodilator component of these biphasic reactions, these two blocking agents were used singly to more clearly delineate the alpha- and beta-receptor properties of dobutamine.

The curves relating vasodilator responses to doses of isoproterenol and doses of dobutamine were parallel; the relative doses required to produce a 50% increase in flow were isoproterenol 1 and dobutamine 180 (Fig. 5). The curves relating vasoconstrictor responses to doses of norepinephrine and doses of dobutamine were not parallel (Fig. 6). Although norepinephrine could reduce flow to zero, maximal doses of dobutamine did not reduce flow below approximately 50% of the control level. At a flow reduction of 30% (linear portion of the dobutamine curve), the relative doses were norepinephrine 1 and dobutamine 14.5. Figure 6 also illustrates that treatment with reserpine had no effect on the vasoconstriction responses. However, further
FIGURE 2
Relationship between the increase in contractile force and the dose of isoproterenol, norepinephrine, or dobutamine in dogs without and with reserpine. Solid symbols indicate reserpine treatment for isoproterenol and norepinephrine experiment and open symbols indicate reserpine treatment for dobutamine experiment. Drugs were administered as intravenous bolus injections to vagotomized dogs anesthetized with sodium pentobarbital.

FIGURE 3
Relationship between the increase in heart rate and the dose of isoproterenol, norepinephrine, or dobutamine without and with reserpine. Solid symbols indicate reserpine treatment for isoproterenol and norepinephrine experiments, and open symbols indicate reserpine treatment for dobutamine experiment. Preparation was the same as that in Figure 2.

treatment with imipramine produced a shift to the right of the vasoconstrictor curves for norepinephrine and dobutamine (Fig. 7).

RENUAL HEMODYNAMICS
Previously established criteria for assigning dopaminelike activity to a drug (12) include an
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FIGURE 4
Relationship between the percent increase in heart rate and contractile force produced by isoproterenol, norepinephrine, and dobutamine. The dogs were anesthetized with sodium pentobarbital and had not been treated with reserpine.

FIGURE 5
Relationship between the increase in femoral artery blood flow and the dose of isoproterenol (squares) or dobutamine (circles) injected via the femoral artery in anesthetized dogs. The femoral and sciatic nerves had been sectioned acutely and phenoxybenzamine (5 mg/kg) had been infused via the femoral artery before the responses were obtained.

observed increase in renal blood flow when the compound is injected into the renal artery, the failure of beta-receptor blockade to antagonize this renal vasodilation, and the failure of the compound to produce an increase in femoral blood flow following beta-receptor blockade. The aforementioned hind-limb studies demonstrated that dobutamine was capable of stimulating both alpha and beta receptors in the vasculature of the hind limb without evidence of nonselective vaso-
dilation. The results observed in the renal vasculature (Fig. 8) demonstrated that following alpha-receptor blockade dobutamine had only minor renal vasodilator activity. The maximum response observed was an 8% increase above control renal blood flow. Subsequent administration of propranolol suppressed this response, indicating that the vasodilator activity was mediated by beta receptors. The relative potency of isoproterenol and dobutamine (at maximum dobutamine response) was isoproterenol 1 and dobutamine 480.

Discussion

Previous reports (1–3) have demonstrated that dobutamine produces positive inotropic effects without inducing the changes in blood pressure characteristic of treatment with norepinephrine or isoproterenol, thereby indicating that dobutamine may be cardioselective. The present experiments were performed to quantify the relative cardiac and peripheral beta-receptor effects of dobutamine and to evaluate peripheral alpha-receptor and dopaminergic activity.

Our results indicated that dobutamine possessed an inotropic efficacy similar to that of isoproterenol or norepinephrine. When the hemodynamic effects of norepinephrine and dobutamine were compared in chloralose-anesthetized dogs with intact baroreceptor reflexes, dobutamine consistently increased cardiac output and decreased total peripheral resistance, whereas norepinephrine produced an increase in total peripheral resistance. As the dose of dobutamine was increased, a positive chronotropic effect began to appear. This response contrasted with the response to norepinephrine—a slight reflex slowing of heart rate. In a second series of studies, the drugs were administered as intravenous bolus injections to vagotomized, pentobarbital-anesthetized dogs with intact carotid baroreceptor reflexes. Results obtained from these studies showed that, for an equivalent inotropic response, the chronotropic response to dobutamine was similar to that to norepinephrine but less than that to isoproterenol (Fig. 3). The relatively greater chronotropic response to isoproterenol was most probably secondary to sympathetic stimulation resulting from a vasodepressor effect of the drug. Experiments in dogs treated with reserpine demonstrated that the inotropic and chronotropic responses to the three amines were mediated by direct effects and not by the release of endogenous norepinephrine. The minor effects of dobutamine on blood pressure in the hemodynamic studies might be at-
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tributable to the peripheral vascular effects defined in the femoral and renal blood flow studies.

In the denervated hind-limb preparations, pilot studies indicated that responses to intra-arterial injections of dobutamine were biphasic with an initial vasoconstriction followed by a subsequent vasodilation. After alpha-receptor blockade, vasodilator curves for isoproterenol and dobutamine were parallel. The fact that the vasodilation produced by both drugs was abolished by propranolol indicates that it was mediated by beta receptor stimulation.

The renal blood flow studies demonstrated that direct intra-arterial injections of dobutamine could increase renal blood flow to a maximum of only 8% above control. This effect was mediated by beta-receptor activation, since it was attenuated by propranolol, as was the response to isoproterenol. The renal blood flow responses to dopamine, on the other hand, were not attenuated by propranolol. Thus, dobutamine failed to demonstrate a selective renal vasodilator activity similar to that of dopamine.

The femoral vasoconstrictor component that was studied after prior blockade of beta-receptor effects was mediated by direct alpha-receptor stimulation. The vasoconstrictor effect of dobutamine was limited, which might have been due to low intrinsic alpha-receptor activity. The effects of both norepinephrine and dobutamine were unaffected by treatment with reserpine and were abolished by phenoxybenzamine. The previously reported finding (8) that the vasoconstrictor effects of dobutamine and tyramine are shifted by imipramine (Fig. 9) does not indicate an indirect mode of action. Catecholamines with large N-substituted radicals, such as dobutamine, are not subjected to uptake into adrenergic nerve endings (13). In the present experiments, the antagonism of the vasoconstrictor effects of dobutamine by imipramine was equivalent to its antagonism of norepinephrine-induced vasoconstriction indicating that the effect of imipramine was mediated by alpha-receptor antagonism (14-16).

We related the cardiac and peripheral beta-adrenergic activities of dobutamine by referring both actions to those of a common agent, isoproterenol. The relative dose of dobutamine producing an equivalent inotropic effect was approximately 43 times that of isoproterenol, but the relative dose producing equivalent femoral and renal vasodilation was 180 and 480 times that of isoproterenol, respectively. Thus, in terms of beta-receptor effects, dobutamine is more cardioselective than is isoproterenol.
In the present experiments, no peripheral vasodilation was produced by high doses of norepinephrine after administration of phenoxybenzamine. The cardiac beta-receptor and peripheral alpha-receptor activities of norepinephrine and dobutamine closely resembled one another, since the molar ratio of norepinephrine to dobutamine for an equivalent effect on heart rate, contractile force, and peripheral vascular alpha-receptor activity was 1:15, 1:12, and 1:14, respectively. Accordingly, the peripheral vasodilator effects of dobutamine relative to its positive chronotropic effects are much greater than those for norepinephrine. Thus, dobutamine lies between isoproterenol and norepinephrine in terms of cardioselectivity and beta-receptor activity; dobutamine is relatively less effective on peripheral beta receptors than is isoproterenol, but it is more effective on peripheral beta receptors than is norepinephrine.

It must be pointed out that the femoral vascular alpha- and beta-receptor effects of dobutamine are most probably additive, since the dose ranges overlap. A comparison of Figures 5 and 6 illustrates that the vasoconstrictor properties of dobutamine become evident at a dose of approximately 1 nmole, but the vasodilator effects do not appear until a dose of approximately 10 nmol. Figure 1 supports the fact that the vasoconstrictor dose-response curve lies to the left of that for vasodilation since the figure shows a slight but significant increase in aortic pressure at lower doses of dobutamine. As the dose is increased (16 μg/kg min⁻¹) peripheral vasodilation becomes dominant and there is a downward trend in aortic pressure and total peripheral resistance. This effect might be explained by again referring to Figures 5 and 6. The vasoconstrictor effect is of limited magnitude and reaches a maximum at approximately 100 nmols. Doses above this level further stimulate the peripheral beta-receptors, resulting in a net increase in femoral blood flow.

Dobutamine appears to be a sympathomimetic amine which produces important systemic hemodynamic effects different from those produced by either isoproterenol or norepinephrine. It differs from isoproterenol in two important ways. With dobutamine (1) femoral and renal vascular beta receptors are affected relatively less than are those of the heart, and (2) direct alpha-receptor effects are produced on resistance vessels. Dobutamine is less effective as a peripheral vasoconstrictor but more effective as a peripheral beta-receptor agonist than is norepinephrine when the two drugs are administered in doses which induce equivalent positive inotropic effects. As a result, dobutamine decreases, rather than increases, total peripheral resistance. This decrease in resistance, coupled with the increase in myocardial contractile force, probably accounts for the increase in cardiac output produced by dobutamine but not by norepinephrine.

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