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DIMETHYL ISOPROPYLMETHOXAMINE: A SELECTIVE β-RECEPTOR BLOCKING AGENT

BY

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The postulate of a dual catechol amine receptive mechanism by Ahlquist (1948) has received widespread support. The validity of this hypothesis is strongly supported by the actions of specific receptor blocking agents. Compounds such as phenoxybenzamine, dibozane, phentolamine and tolazoline will block α-receptors but not β-receptors (Levy & Ahlquist, 1960; Levy & Ahlquist, 1961; Levy & Tozzi, 1963). Compounds such as dichloroisoprenaline (Powell & Slater, 1958; Moran & Perkins, 1958; Ahlquist & Levy, 1959; Levy, 1959), pronethalol (Black & Stephenson, 1962), propranolol, (Black, Crowther, Shanks & Dornhorst, 1964), MJ-1998 and MJ-1999 (Lish, Weikel & Dungan, 1965; Stanton, Kirchgessner & Parmenter, 1965; Kvam, Riggilo & Lish, 1965) will block β-receptors but not α-receptors. Most α-receptor blocking agents will block all α-receptor sites and most β-receptor blocking agents will block all β-receptor sites. However, several compounds have recently been described as capable of producing selective blockade of some but not all β-receptor sites. *N*-Isopropylmethoxamine will reduce the hyperglycaemia and increase in free fatty acids due to adrenaline (Burns, Colville, Lindsay & Salvador, 1964; Salvador, Colville, April & Burns, 1964). We have found that, while *N*-isopropylmethoxamine will block the inhibitory responses of the rat isolated uterus preparation to isoprenaline, it has no effect on the positive inotropic, positive chronotropic, intestinal inhibitory, or femoral blood flow responses to isoprenaline (Levy, 1964). *N*-Tertiary butylmethoxamine (butoxamine) has been reported to produce a blockade of the hyperglycaemia and increased mobilization of free fatty acids produced by adrenaline and isoprenaline (Salvador & April, 1965; Burns & Lemberger, 1965). We have found that butoxamine will produce a selective blockade of some but not all β-receptor sites (Levy, 1966).

The purpose of this study was to determine the effects of 1-(2′,4′-dimethylphenyl)-2-isopropylamino-1-propanol hydrochloride, a compound that resembles *N*-isopropylmethoxamine and butoxamine structurally, on catechol amine receptors.

METHODS

Experiments with dogs. Adult mongrel dogs, of either sex, were used. In experiments which included measurement of intestinal motility, the anaesthetic regimen consisted of the subcutaneous injection of 10 mg/kg of morphine sulphate followed in 30 min by the intravenous injection of 20 mg/kg of pentobarbitone sodium. In all other dog experiments the animals were anaesthetized

with pentobarbitone sodium, 15 mg/kg, and barbitone sodium, 220 mg/kg, given together intravenously. The trachea, a carotid artery and a jugular vein were cannulated. To record intestinal motility, a loop of ileum was exposed through a short midline incision; a water-filled balloon, 5 to 10 ml. in capacity, connected by a flexible catheter to a Statham transducer, was inserted into the ileum through a stab wound. Arterial pressure was recorded by a Statham transducer from a carotid artery. Heart rate was continuously recorded with a linear electronic tachometer, triggered by the electrocardiogram. Cardiac contractile force was measured with the animals under positive pressure artificial ventilation. After bilateral vagotomy, the heart was exposed by a thoracotomy, and a strain-gauge arch (Boniface, Brodie & Walton, 1953; Cotten & Bay, 1956) was sutured to the right ventricle. Femoral arterial flow was recorded with a square-wave electromagnetic flowmeter (Model 301, Carolina Medical Electronics). Non-cannulating flow probes with a diameter of 5, 7 or 10 mm were used, depending upon the size of the vessel. Drugs were injected intra-arterially into a small branch of the femoral artery that was cannulated with a fine polyethylene cannula (PE-10). In this manner femoral arterial flow was not interrupted. All other drug injections were made into the jugular vein. Recordings were made with a multichannel cathode-ray tube camera system (Electronics for Medicine, Model DR-8).

Experiments with rat isolated uterine segments. Mature Sprague-Dawley rats, weighing 150 g or more, were used in all *in vitro* studies. Uterine segments were suspended in a 10 ml. organ-bath at a constant temperature of 38° C. Locke solution (g/100 ml.: NaCl 0.9, KCl 0.042, $CaCl_2$ 0.024, glucose 0.1 and $NaHCO_3$ 0.05), bubbled with 95% oxygen and 5% carbon dioxide, was used in all studies. Spontaneous motility was recorded isotonically by means of a linear motion transducer (Phipps & Bird, Model ST-2), or isometrically by means of a force displacement transducer (Statham). All agonists were allowed to act for 3 min before adding the antagonist. Recording was with the system described above.

Drugs. (−)-Adrenaline bitartrate, (±)-isoprenaline hydrochloride, (−)-phenylephrine hydrochloride and (−)-noradrenaline were prepared daily from stock solutions with a concentration of 1 mg/ml. Ethylnoradrenaline hydrochloride was prepared as a 5 mg/ml. solution. The stock solutions were made using 0.9% saline containing 1 mg/ml. sodium bisulphite as a preservative. 1-(2′,4′-Dimethylphenyl)-2-isopropylamino-1-propanol hydrochloride (dimethyl isopropylmethoxamine) was prepared as a 10 mg/ml. solution in 0.9% saline. This compound has two asymmetric centres which suggests that four isomers may exist. The present method of synthesis does not allow us to state which isomer is the most active one. Until these synthesis problems are resolved we must state that all results described in this paper are due to a mixture of up to four isomers. Doses of all drugs are expressed in terms of their salts.

RESULTS

The effects of dimethyl isopropylmethoxamine on mean arterial pressure, heart rate, and intestinal motility in the anaesthetized, atropine-treated dog. Dimethyl isopropylmethoxamine was administered in a dose range of 1–10 mg/kg to a group of four dogs. It produced no consistent effect on intestinal motility or heart rate. Rapid injection of 1 to 3 mg/kg or more produced a transient fall in blood pressure (Figs. 1B, 2B). This transient vasodepressor response could be minimized by the slow intravenous infusion of the drug over a 10–15 min interval. After the injection of 3 mg/kg of dimethyl isopropylmethoxamine, the depressor response to ethylnoradrenaline (50 μg/kg) was converted to a pressor response. The pressor response to adrenaline was increased in both size and duration. After 10 mg/kg of dimethyl isopropylmethoxamine, the pressor responses to adrenaline (2 μg/kg), noradrenaline (2 μg/kg) and phenylephrine (20 μg/kg) were also increased, particularly in duration. Unlike *N*-isopropylmethoxamine and *N*-tertiary butylmethoxamine, each of which converted the depressor response to isoprenaline to

a pressor response (Levy, 1964 ; Levy, 1966), dimethyl isopropylmethoxamine did not reverse the depressor response to isoprenaline. The intestinal inhibitory responses to adrenaline, isoprenaline, noradrenaline and phenylephrine were not reduced by dimethyl isopropylmethoxamine in any dosage. The positive chronotropic response to isoprenaline was not appreciably reduced. Some of these results are illustrated in Fig. 1.

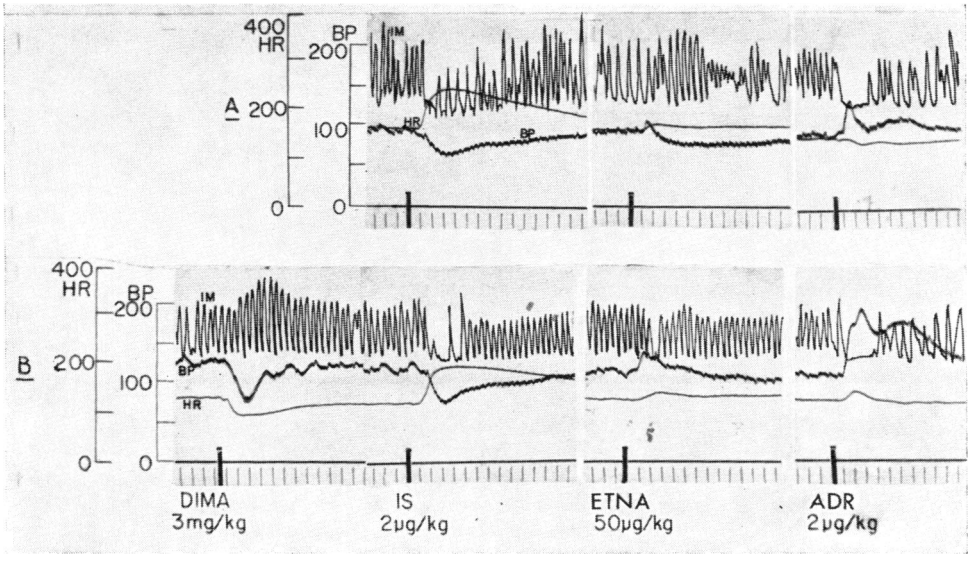

Fig. 1. Effects of dimethyl isopropylmethoxamine (DIMA) on response to isoprenaline (IS), ethylnoradrenaline (ETNA) and adrenaline (ADR) in a dog anaesthetized with morphine-pentobarbital and pretreated with atropine. From above downward, in each panel: Intestinal motility (IM); mean arterial pressure (BP, calibration in mm Hg); heart rate (HR, calibration in beats/min). (A) Control responses to IS (2 µg/kg), ETNA (50 µg/kg) and ADR (2 µg/kg). (B) Responses to the same doses of IS, ETNA and ADR after DIMA (3 mg/kg). Time marks, 10 sec.

The effects of dimethyl isopropylmethoxamine on myocardial contractile force in the dog. In addition to recording contractile force by means of a strain gauge sutured to the right ventricle, mean arterial pressure and heart rate were recorded simultaneously. Dimethyl isopropylmethoxamine was administered in a dose range of 1 to 10 mg/kg to three dogs. After 1 mg/kg there occurred only a transient fall in blood pressure and a decrease in heart rate. However, 3 mg/kg produced a marked reduction in cardiac contractile force as well as a fall in blood pressure and a reduction in heart rate (Fig. 2B); 10 mg/kg produced even greater reductions in the three parameters. The positive inotropic and chronotropic responses to adrenaline, isoprenaline and noradrenaline (2 µg/kg, intravenously) were not greatly reduced by previous treatment with dimethyl isopropyl-methoxamine (Fig. 2).

The effects of dimethyl isopropylmethoxamine upon femoral arterial flow. The intra-arterial administration of dimethyl isopropylmethoxamine in doses of 10, 30 and 100 µg/kg produced only an increase in femoral arterial flow. This suggests that the drug possesses an intrinsic peripheral vasodilator effect (Fig. 3A). The increased femoral

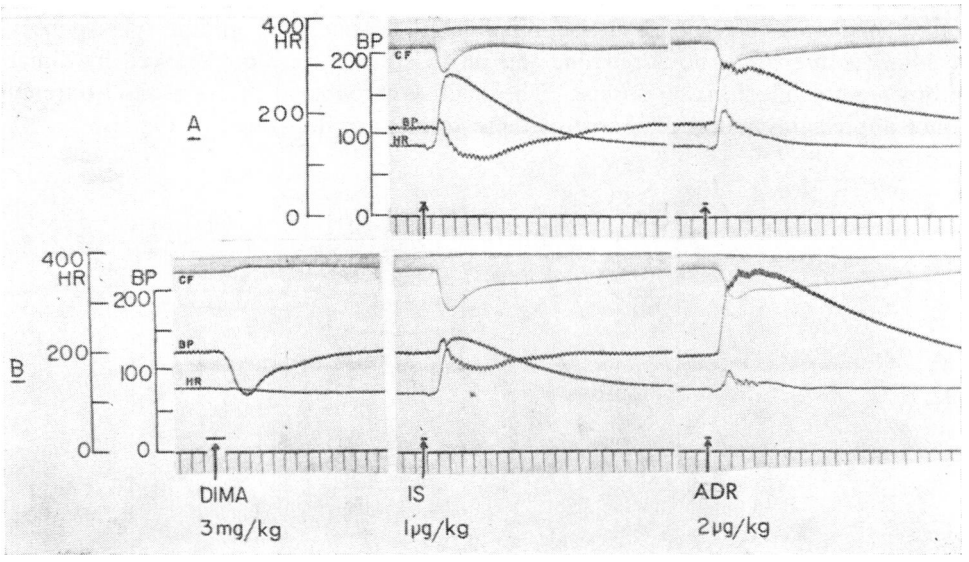

Fig. 2. Effects of dimethyl isopropylmethoxamine (DIMA) on responses to isoprenaline (IS) and adrenaline (ADR) in a dog anaesthetized with pentobarbitone-barbitone. From above downward, in each panel: myocardial contractile force (CF); mean arterial pressure (BP, calibration in mm Hg) and heart rate (HR, calibration in beats/min). (A) Control responses to IS (1 μg/kg) and ADR (2 μg/kg). (B) Responses to the same doses of IS and ADR after DIMA (3 mg/kg). Time marks, 10 sec.

flow in response to 100 μg/kg was not appreciably reduced by previous intravenous injection of atropine (1 mg/kg), chloropheniramine (4 mg/kg) or pronethalol (4 mg/kg) (Fig. 3A).

The intra-arterial injection of isoprenaline (0.1 μg/kg) produced a marked but transient increase of femoral flow that suggested a local vasodilator action. The intra-arterial injection of ethylnoradrenaline (5 μg/kg) produced a marked but transient increase in femoral flow, often preceded by a brief reduction in flow which suggested an initial phase of vasoconstriction. After treatment with 3 mg/kg of dimethyl isopropylmethoxamine, the increase in femoral flow due to isoprenaline (0.1 μg/kg) was markedly reduced. The response to ethylnoradrenaline was " reversed " to a reduction in femoral flow (Fig. 4) that resembled the vasoconstrictor response to intra-arterial adrenaline. This same reversal of the blood flow response to ethylnoradrenaline could be produced by previous treatment with 0.1 mg/kg of dimethyl isopropylmethoxamine given intra-arterially; this treatment itself had no appreciable inhibitory effect on the femoral flow response to 0.1 μg/kg of isoprenaline and no larger dose of dimethyl isopropylmethoxamine was given intra-arterially.

The effects of dimethyl isopropylmethoxamine on the rat isolated uterus preparation. All results described here were obtained in a minimum of five spontaneously contracting uterine segments. The rats were not primed with oestrogen because we have previously reported (Levy & Tozzi, 1963) that the relationship between adrenergic agonists and

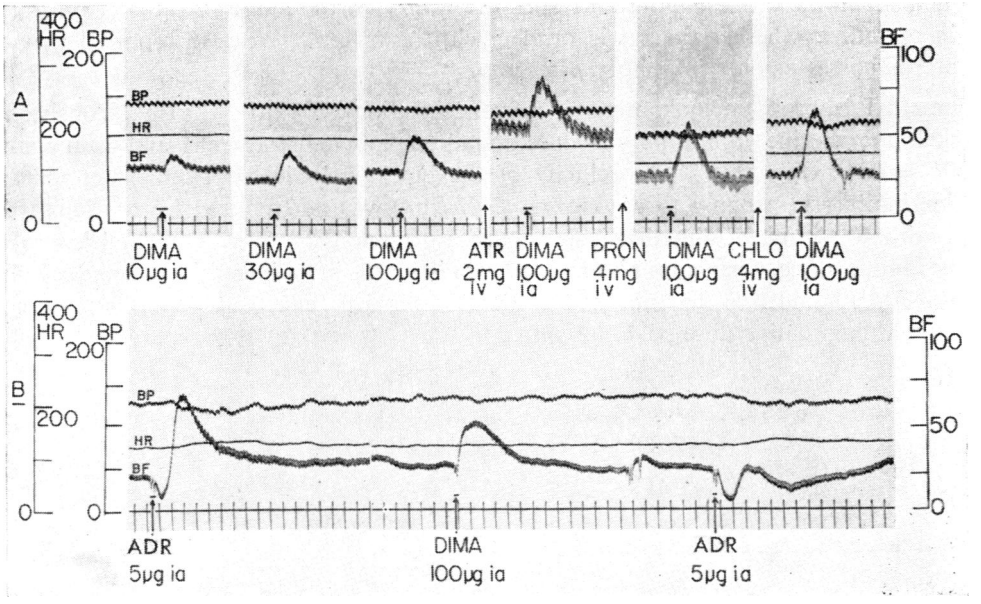

Fig. 3. Effects of dimethyl isopropylmethoxamine on mean arterial pressure, heart rate and femoral flow in two dogs anaesthetized with pentobarbitone-barbitone. From above downward, in each panel: Mean arterial pressure (BP, calibration in mm Hg), heart rate (HR, calibration in beats/min) and femoral flow (BF, calibration in ml./min). (A) Dog 1: control responses to DIMA (10, 30 and 100 μg/kg, intra-arterially). Responses to DIMA (100 μg/kg, intra-arterially) before and after atropine sulphate (ATR, 2 mg/kg, intravenously), pronethalol (PRON, 4 mg/kg, intravenously) and chloropheniramine (CHLO, 4 mg/kg, intravenously). (B) Dog 2: response to adrenaline (ADR 5 μg/kg, intra-arterially) before and after DIMA (100 μg/kg, intra-arterially). Time marks, 10 sec.

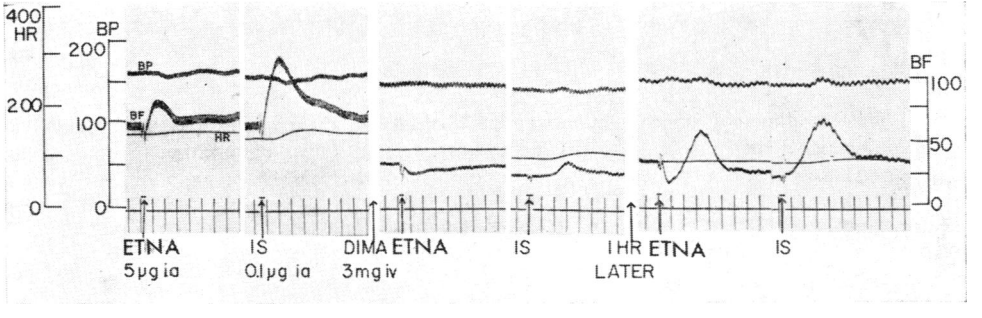

Fig. 4. Effects of dimethyl isopropylmethoxamine (DIMA) on responses to ethylnoradrenaline (ETNA) and isoprenaline (IS) in a dog anaesthetized with pentobarbitone-barbitone. From above downward: Mean arterial pressure (BP, calibration in mm Hg); femoral flow (BF, calibration in ml./min) and heart rate (HR, calibration in beats/min). Responses to ETNA (5 μg/kg, intra-arterially) and IS (0.1 μg/kg, intra-arterially) before and after DIMA (3 mg/kg, intravenously). Time marks, 10 sec.

antagonists on the rat isolated uterus does not vary with the phase of the oestrous cycle, strain of rat, or whether the rat is primed with oestrogen. We also reported that the rat uterus possesses only inhibitory β-receptors.

Dimethyl isopropylmethoxamine, in a concentration of 1 to 5 μg/ml., consistently blocked the uterine inhibitory response to adrenaline (0.01 μg/ml.) and isoprenaline (0.001 μg/ml.) (Fig 5). The specificity of the block is indicated by the fact that the uterine inhibitory response to papaverine (3 μg/ml.) was not reduced by previous treatment with 40 μg/ml. of dimethyl isopropylmethoxamine (Fig. 5B). Dimethyl isopropylmethoxamine alone, when administered at 3 min intervals in an increasing dosage schedule of 10, 30, 50 and 100 μg/ml., had a weak inhibitory effect in doses in excess of 30 μg/ml. (cumulative dose of 40 μg/ml.).

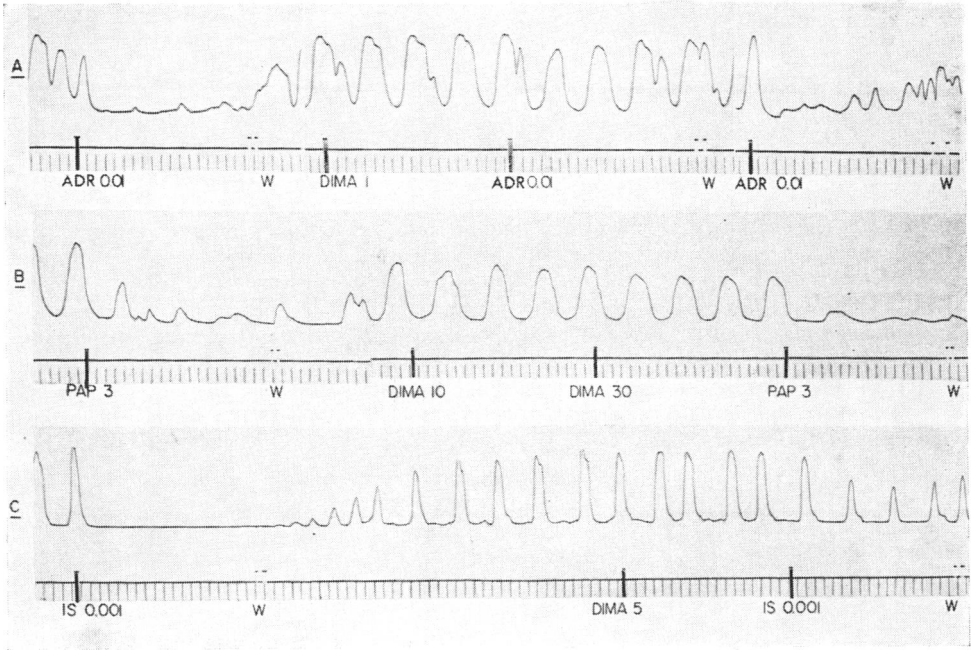

Fig. 5. Effects of dimethyl isopropylmethoxamine (DIMA) on the responses to adrenaline (ADR), papaverine (PAP) and isoprenaline (IS) on the rat isolated uterus preparation (isotonic contractions. (A) Responses to ADR (0.01 μg/ml.) before and after DIMA (1 μg/ml.). (B) Responses to PAP (3 μg/ml.) before and after DIMA (40 μg/ml.). (C) Responses to IS (0.001 μg/ml.) before and after DIMA (5 μg/ml.). Time marks, 10 sec.

The blocking agent action of dimethyl isopropylmethoxamine was completely reversible as indicated by the return of the uterine inhibitory responses to the agonists after washing. We have also previously reported that dichloroisoprenaline inhibits uterine motility presumably by first stimulating β-receptors (Levy & Tozzi, 1963). In this study, 5 μg/ml. of dimethyl isopropylmethoxamine prevented the uterine inhibitory response to dichloroisoprenaline (0.1 μg/ml.).

DISCUSSION

The currently accepted concept of the adrenergic receptive mechanism was suggested by Ahlquist in 1948. This hypothesis attempts to classify the actions of the catechol amines according to their ability to activate either α- or β-receptors or both. Part of the original evidence for this concept was based upon the ability of adrenergic-blocking agents such as phenoxybenzamine and phentolamine to block the effects of activation of α-receptors without producing any significant blocking effect on β-receptors. The introduction of the dichloro analogue of isoprenaline (Powell & Slater, 1958), provided a compound that could selectively block the responses to β-receptor activation and not those to α-receptor activation. It is now usual to place adrenergic-blocking agents in two distinct classes and to refer to them as α- or β-blocking agents.

We have recently described the adrenergic-blocking activity of *N*-isopropylmethoxamine (Levy, 1964) and tertiary butylmethoxamine (Levy, 1966), compounds that selectively block some but not all β-receptors. *N*-Isopropylmethoxamine can block the hyperglycaemic and increased free fatty acid responses to catechol amines (Burns *et al.*, 1964; Salvador *et al.*, 1964). The same blocking effects are also produced by a close analogue of *N*-isopropylmethoxamine, tertiary butylmethoxamine (butoxamine) (Burns & Lemberger, 1965; Salvador & April, 1965). Aside from its metabolic blocking action, *N*-isopropylmethoxamine blocks only the β-receptors in the rat uterus. The positive inotropic and chronotropic, intestinal inhibitory and vasodilator responses to isoprenaline are not blocked by *N*-isopropylmethoxamine (Levy, 1964). Tertiary butylmethoxamine has a somewhat similar restricted blocking effect on β-receptors and differs from *N*-isopropylmethoxamine in that it also blocks the vasodilator response to isoprenaline in the femoral vascular bed of the dog.

Dimethyl isopropylmethoxamine resembles tertiary butylmethoxamine in its selective blockade of some but not all β-receptor sites. The rat uterine inhibitory response to isoprenaline as well as the increase in femoral vascular flow after intra-arterial injection are blocked by dimethyl isopropylmethoxamine.

The femoral flow response to intra-arterial ethylnoradrenaline, which usually is one of transient vasoconstriction followed by more prolonged vasodilation, is converted to a purely vasoconstrictor response. If we consider the fact that ethylnoradrenaline can activate both α- and β-receptors, then blockade of β-receptors by dimethyl isopropylmethoxamine would unmask the stimulant effect of ethylnoradrenaline on α-receptors resulting in vasoconstriction. These blood flow studies indicate that dimethyl isopropylmethoxamine selectively blocks β-receptors in the femoral vascular bed. Dimethyl isopropylmethoxamine does not block the positive inotropic or chronotropic responses to isoprenaline even after doses (3 to 10 mg/kg) that block the femoral flow responses to isoprenaline.

Rather than postulate the existence of an additional type of receptor, we feel that dimethyl isopropylmethoxamine should be added to a new and growing group of agents that selectively block some but not all β-receptors. It is interesting to note that all of these selective β-blocking agents possess an α-methyl group. The addition of an α-methyl group to dichloroisoprenaline, a "classical" β-blocking agent, results in a compound that possesses the same selectivity of β-receptor blockade as dimethyl isopropylmethox-

amine in that the femoral flow responses to isoprenaline are reduced but the positive inotropic and chronotropic responses are not (Van Deripe & Moran, 1965).

SUMMARY

1. The adrenergic blocking activity of 1-(2′,4′-dimethylphenyl)-2-isopropylamino-1-propanol hydrochloric acid (dimethyl isopropylmethoxamine) was determined in the anaesthetized dog and the rat isolated uterus preparation.

2. Dimethyl isopropylmethoxamine reduced the vasodilator response to isoprenaline, given intra-arterially, in the femoral vascular bed. The biphasic constrictor-dilator response to ethylnoradrenaline, given intra-arterially, was converted to a purely vasoconstrictor response.

3. Dimethyl isopropylmethoxamine produced no significant blockade of the positive inotropic, positive chronotropic or intestinal inhibitory responses to adrenaline or isoprenaline in the anaesthetized dog.

4. Specific blockade of the inhibitory β-receptors in the rat isolated uterus preparation was produced by dimethyl isopropylmethoxamine.

5. It is concluded that dimethyl isopropylmethoxamine can selectively block some but not all β-receptor sites.

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