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EFFECTS OF COMPOUND MJ-1988 ON MYOCARDIAL CONTRACTILITY, OXYGEN CONSUMPTION, CORONARY BLOOD FLOW AND VASCULAR RESISTANCE

BY

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Preliminary reports have shown that 6,7-dimethoxy-4-ethylquinazoline, known as MJ-1988, possesses potent cardiac stimulating, bronchodilator and vasodilating properties (Lish, Cox, Dungan & Robbins, 1964). A drug with this combination of pharmacological effects might have important therapeutic possibilities. This study was undertaken to assess the potency of an effective dose of the compound on the contractility of the working canine left ventricle. The experimental preparation used also allowed the assessment of coronary blood flow, myocardial oxygen consumption and vascular resistance.

METHODS

Experiments were performed in adult mongrel dogs, weighing 10.5 to 17 kg. The dogs were anaesthetized by an intravenous infusion of a warmed solution of urethane (600 mg/kg) and chloralose (60 mg/kg). The rate and depth of respiration was controlled by a Bird Mark 8 positive pressure respirator. The chest was opened through a median sternotomy and the working left heart model was prepared as follows (Fig. 1). After preliminary dissection, haemostasis, and isolation of a femoral artery, heparin, 3 mg/kg, was administered, followed by 30 mg every hour. The superior and inferior vena cavae were cannulated and the azygos vein divided. The caval return was led to a reservoir (Res. 1) by siphon drainage. From the reservoir the blood drained by gravity to a bubble oxygenator. Oxygenated blood, warmed to 37° C, was returned *via* a roller pump (P) through a Shipley-Wilson rotameter (R1) to the main pulmonary artery. A ligature placed around the cannulated pulmonary artery thus completely isolated the right heart so that it received only coronary venous drainage. The total coronary flow (less left ventricular Thebesian flow) was led from the cannulated right heart through a rotameter (R2) to the reservoir (Res. 1). This rotameter was calibrated during each experiment by timed volumetric collection of coronary venous blood flow.

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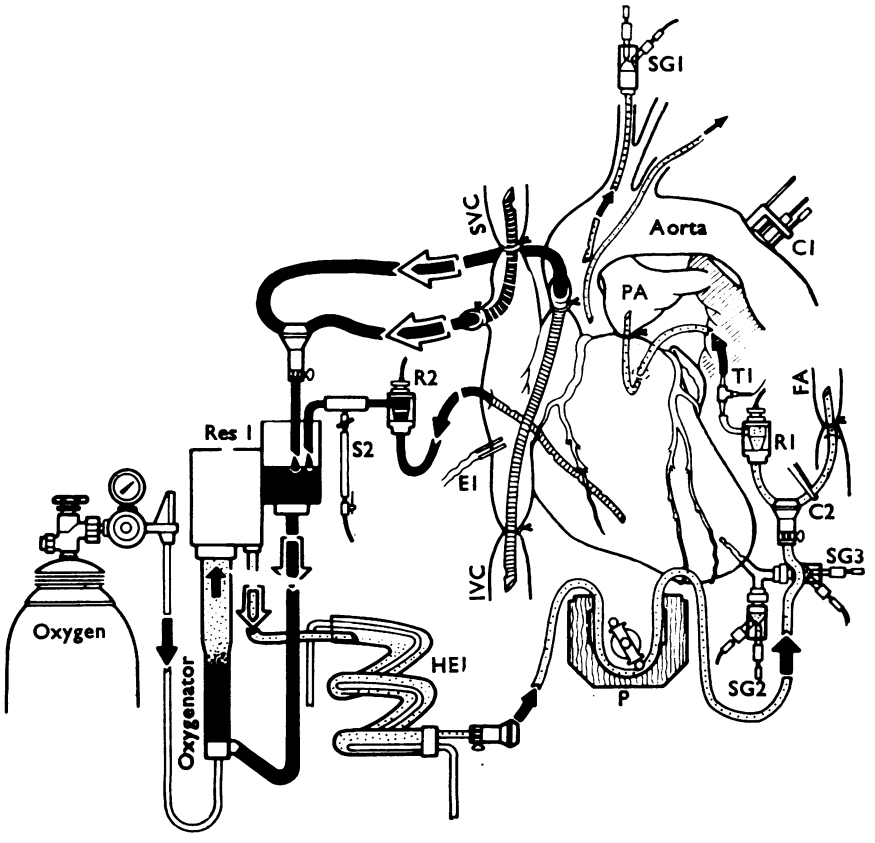

Fig. 1. Right heart bypass preparation. See text for description. Res. 1 = venous return reservoir, HE1 = heat exchanger, P = roller pump, R1 = cardiac input rotameter, T1 = temperature probe, R2 = total coronary flow rotameter, S2 = coronary venous blood sampling site, SG1 = aortic pressure strain gauge, SG2 = left ventricular pressure strain gauge, SG3 = left ventricular diastolic pressure strain gauge, C1 = aortic screw clamp, C2 = bypass selector clamp, E1 = right atrial bipolar pacing electrode, FA = femoral artery. Coronary arterial sampling site is the catheter at the aortic valve.

Aortic pressure was controlled by adjusting a screw clamp placed about the descending aorta distal to the left subclavian artery. Pressures were measured in the aorta (SG1) through a short polyethylene cannula introduced into the left internal mammary artery. Left ventricular pressure and left ventricular diastolic pressure were measured with strain gauges (SG2, SG3) on each arm of a Y-shaped cannula placed in the left ventricle through the apical dimple. All pressures were measured with Statham P-23db strain gauges.

Following sino-atrial node crush, heart rate was controlled by electrical pacing (Grass stimulator Model S-4 through a Grass isolation unit SIU-4B) on the right atrium (E1).

The rate of rise of left-ventricular pressure (dp/dt) was recorded electronically by differentiating the output of the coupler recording left ventricular pressure. Calibration of dp/dt was accomplished by feeding a linear triangle wave (Hewlett-Packard function generator model 202A) of known slope and amplitude into the differentiator through the coupler in which left ventricular pressure was recorded.

The standard electrocardiogram was recorded in the usual manner. All variables were continuously recorded on an eight-channel Beckman, Type S-II, direct writing oscillograph.

The femoral artery (FA) was also cannulated. A "Y" connector was used so that a line from the output of the roller pump led to both the femoral artery and the pulmonary artery. Thus by switching a clamp from the femoral artery line to the pulmonary line we could establish total cardiopulmonary bypass at will.

The same preparation was used in six cardiac denervated dogs. Five of these dogs had auto-transplantation of the heart from 1 to 3 months before the experiments (Willman, Cooper, Cian & Hanlon, 1962). One dog had cardiac denervation by mediastinal neural ablation 3.5 months before the experiment (Cooper, Gilbert, Bloodwell & Crout, 1961).

Experimental procedure

After the animal was prepared, adequate time was allowed to obtain a steady state. Ventricular function curves were determined by changing the flow load and a shift of the curve to the left indicated an increase in contractility.

Compound MJ-1988 was administered by constant infusion into the pulmonary artery with a Harvard infusion pump (model 600–900 V) at 0.375 mg/kg/min following a steady state control tracing. This dose was chosen since it consistently demonstrated an effect and this effect was not particularly enhanced by higher dosage. Also this dose avoided an arrhythmic effect sometimes observed at higher dosage. Other experiments have shown that anaesthetized dogs receiving 1 mg/kg/min can tolerate up to 40 mg/kg of Compound MJ-1988 before signs of cardiovascular insufficiency are seen (Lish *et al.*, 1964).

We used changes in the following parameters as indices of left ventricular contractility: left ventricular end-diastolic pressure (LVEDP); the maximum rate of rise of left ventricular pressure (maximum dp/dt), the duration of systole and ventricular function curves. With afterload, systemic flow, and heart rate constant, an increase in contractility occurred if there was a fall in left ventricular end-diastolic pressure, an increase in maximum dp/dt, a shortening of the duration of systole, and a shift to the left in the ventricular function curve.

The same procedure was carried out following β-receptor blockade with Compound MJ-1999 (Lish, Weikel & Dungan, 1965) in both normal and cardiac denervated dogs. Cardiac response to (±)-isoprenaline (10 μg) injected into the pulmonary artery was determined before and after the administration of MJ-1999 to verify the block.

In four dogs total cardiopulmonary bypass was established and the output from the pump held constant. With these circumstances a fall in aortic pressure was the result of a decrease in peripheral vascular resistance. The effect of the addition of MJ-1988 to the venous reservoir was recorded. This was done in two animals after α-receptor blockade with phenoxybenzamine (1 mg/kg) and β-receptor blockade with Compound MJ-1999 (5 mg/kg).

Calculations

Stroke work (Sarnoff & Berglund, 1954) and the duration of systole (Wallace, Mitchell, Skinner & Sarnoff, 1963) were determined as has been described. Myocardial oxygen consumption was determined in eight dogs before and during the administration of MJ-1988 by multiplying total coronary flow times coronary arteriovenous oxygen difference, the component values having been obtained during a steady state of at least 30 sec. The oxygen content was determined on an F & M blood gas analyser, Model 450. This is a gas chromatographic technique in which oxygen content of blood samples is measured chemically in a manner similar to the Van-Slyke method. Haematocrits did not change significantly during the course of the studies.

RESULTS

Effect on myocardial contractility

Figure 2 is a tracing from a typical experimental run. There was a definite fall in left ventricular end-diastolic pressure, a rise in maximum dp/dt and a shortening of

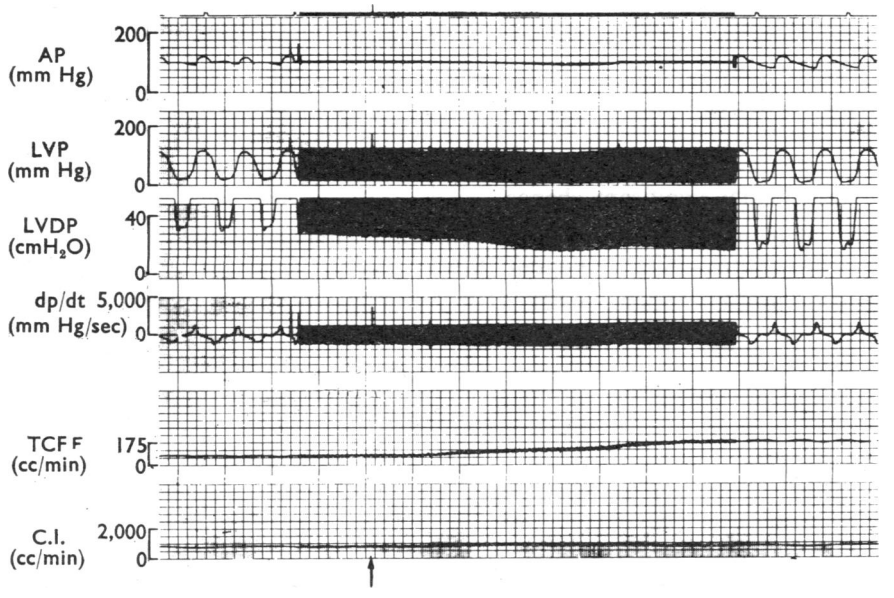

Fig. 2. Effect of Compound MJ-1988 on ventricular contractility and coronary blood flow at constant aortic pressure, cardiac input and heart rate. At the arrow, infusion of Compound MJ-1988, 0.375 mg/kg/min into the pulmonary artery caused a fall in LVEDP, a rise in maximum dp/dt, and an increase in total coronary flow (TCF). AP=aortic pressure, LVP=left ventricular pressure, LVDP=left ventricular diastolic pressure, dp/dt=rate of rise of left ventricular pressure, TCF=total coronary flow, CI=cardiac input.

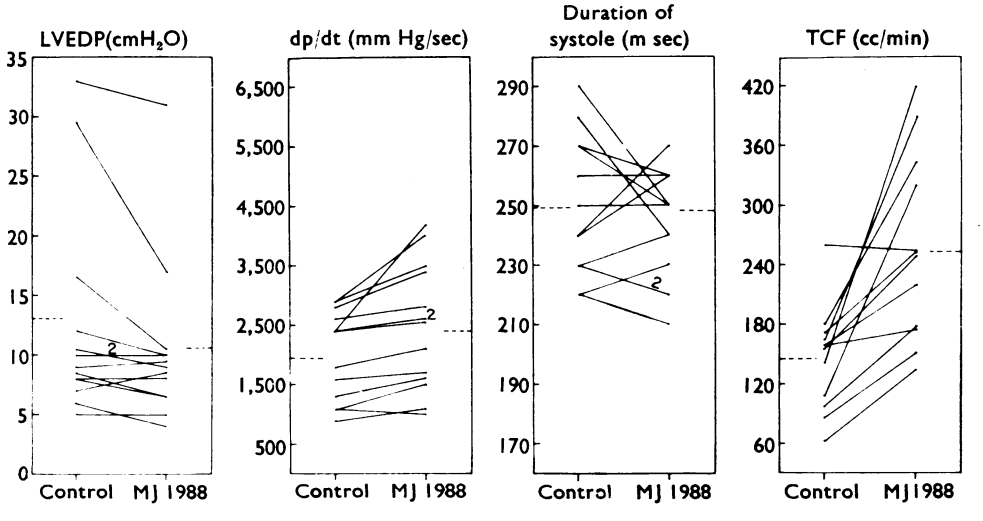

Fig. 3. Changes of indices of contractility in normal dogs upon infusion of Compound MJ-1988, 0.375 mg/kg/min, into the pulmonary artery. The means for each group are represented by the broken lines at the edge of the panels. When a number is present on a line, it denotes the number of observations for the values recorded.

the duration of systole of 20 msec. The results of fourteen such experiments on nine normal dogs are summarized in Fig. 3. The cumulative results show a mean fall in left ventricular end-diastolic pressure from 13.2 ± 2.38 mm Hg to 10.4 ± 1.72 mm Hg ($P<0.05$) and an increase in mean maximum dp/dt from $1,987 \pm 186$ mm Hg/sec to $2,437 \pm 284$ mm Hg/sec ($P<0.005$). There was a decrease in the duration of systole in seven instances, an increase in four, and no change in two. This did not prove to be a statistically significant change.

Figure 4 demonstrates representative ventricular function curves in two experiments. In four animals there was a definite shift to the left with the administration of the compound as in the left-hand panel. Three animals showed a response similar to that in the right-hand panel—that is, there was no essential change in the curve.

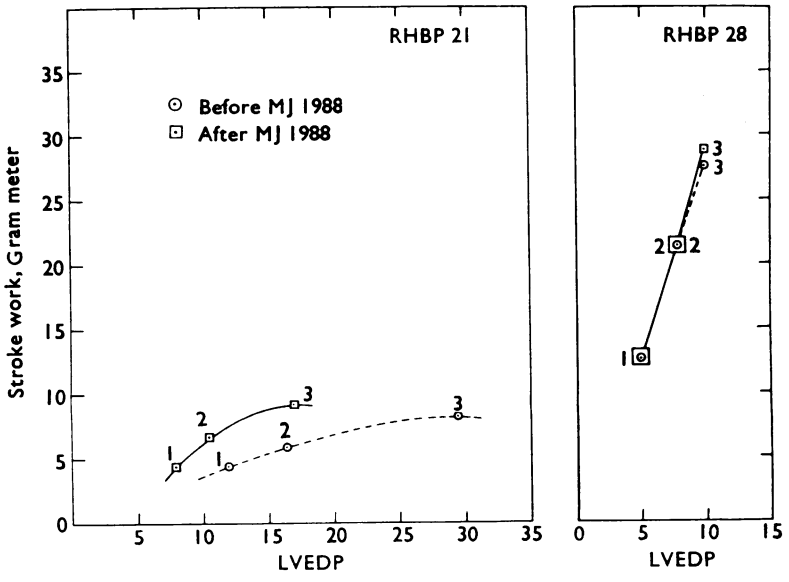

Fig. 4. Effect of infusion of Compound MJ-1988, 0.375 mg/kg/min, into the pulmonary artery, on ventricular function curves (stroke work versus left ventricular end diastolic pressure (LVEDP)), performed by increasing cardiac output stepwise while aortic pressure and heart rate were held constant. Note the more pronounced effect of MJ-1988 on the heart with the lesser baseline contractility.

In order to eliminate the possibility that the effect of the drug was mediated by adrenergic nerve stimulation or by central nervous system influences the drug was administered after total cardiac denervation by autotransplantation of the heart or by mediastinal neural ablation. Denervation is accompanied by total myocardial catecholamine depletion (Cooper, Willman, Jellinek & Hanlon, 1962).

Figure 5 shows the cumulative results of fifteen experiments in six cardiac denervated dogs. There was a mean increase in the maximum dp/dt from $2,666 \pm 296$ to $3,326 \pm 348$ mm Hg/sec ($P<0.001$) and a mean decrease in the duration of systole from 246.4 ± 5.61 to 227.8 ± 4.87 msec ($P<0.001$). There was not a consistent decrease in left ventricular

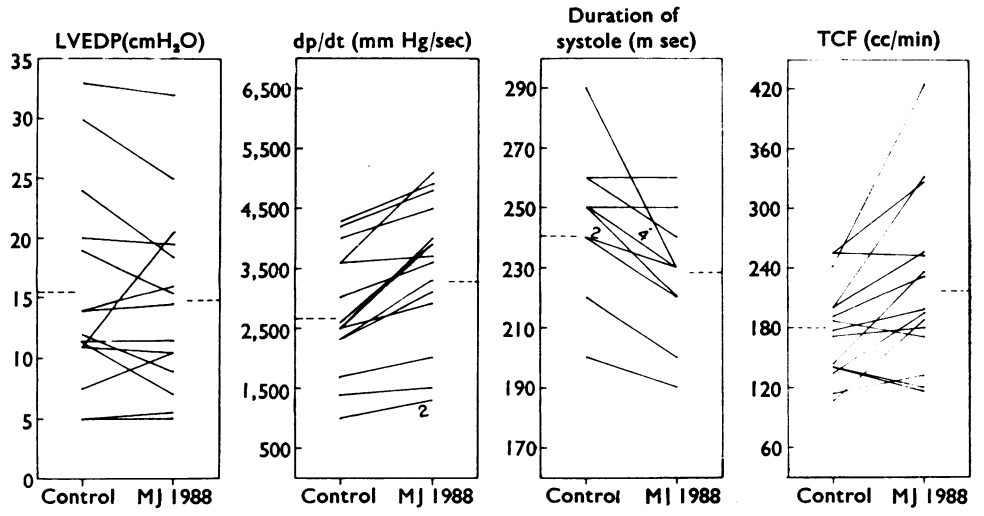

Fig. 5. Changes of indices of contractility in cardiac denervated dogs upon infusion of Compound MJ-1988, 0.375 mg/kg/min, into the pulmonary artery. Notations are the same as those of Fig. 3.

end-diastolic pressure following the administration of MJ-1988 in the cardiac denervated dog, although in many instances there was a fall. In each instance where there was no change or an increase in left ventricular end-diastolic pressure there was an increase in maximum dp/dt and a shortening of the duration of systole.

Effects of β-receptor blockade

To eliminate further the likelihood that Compound MJ-1988 produced its effect through participation of stored or circulating adrenergic amines the effect of Compound MJ-1988 was tested in normal and cardiac denervated dogs following β-receptor blockade with Compound MJ-1999, 1 to 5 mg/kg. Figure 6 is a tracing of such an experiment in a normal dog. The drug was administered following β-receptor blockade in five experiments on the normal dog and three in the cardiac denervated dog. Each time there was clear-cut evidence of a positive inotropic effect.

Effects on coronary blood flow and myocardial oxygen consumption

As was seen on the tracings from the experimental runs (Figs. 2 and 6) there was an increase in coronary blood flow, at times to a striking degree, with the administration of Compound MJ-1988.

Figure 3 (panel on right) summarizes the changes observed in coronary blood flow in the nine normal dogs. All determinations were at constant aortic pressure, heart rate and cardiac input. In the twelve determinations there was a mean increase in coronary flow from 140.4 ± 16.1 to 252.9 ± 29.2 ml./min ($P<0.05$).

Figure 5 (panel on right) summarizes the changes observed in coronary blood flow in fifteen determinations on the six cardiac denervated dogs. The mean increase in flow was from 180.5 ± 13.5 to 225.3 ± 22.9 ml./min ($P<0.01$). The administration of

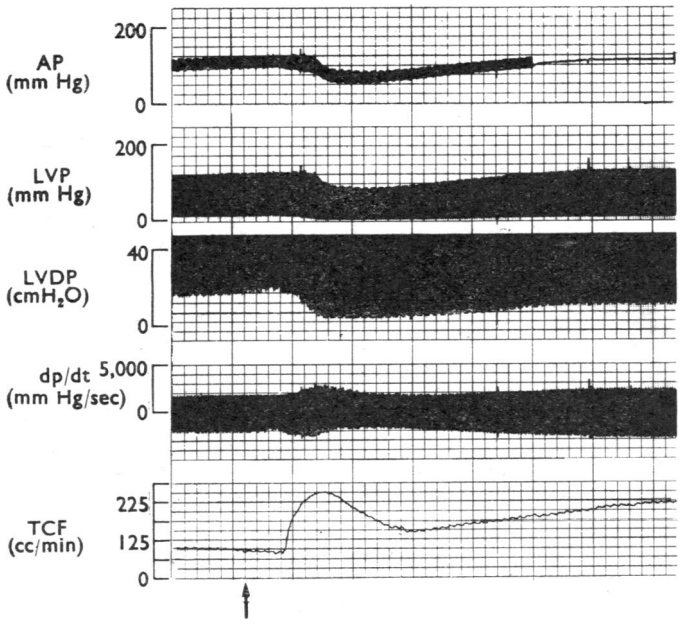

Fig. 6. Effect of Compound MJ-1988 after β-receptor blockade with Compound MJ-1999. Arrow indicates the start of infusion of MJ-1988, 0.375 mg/kg/min, into the pulmonary artery. Note lowered LVEDP and increased maximum dp/dt and coronary blood flow (TCF) after re-establishment of mean aortic pressure (MAP) to pre-infusion level. Also note initially increased maximum dp/dt in face of decreased MAP and LVEDP.

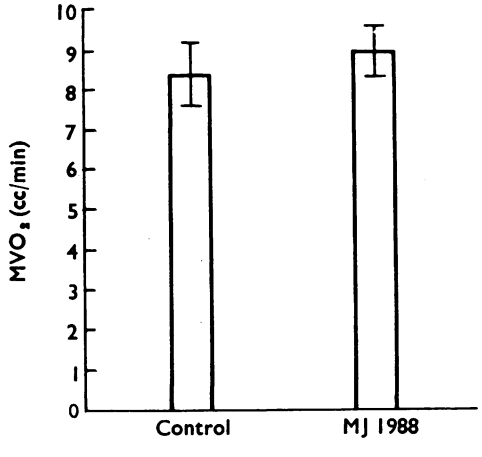

Fig. 7. Effect of infusion of Compound MJ-1988, 0.375 mg/kg/min, into the pulmonary artery, on myocardial oxygen consumption (MVO_2). Bars represent the mean value, line represents standard error of the mean.

MJ-1988 resulted in an increased coronary blood flow in both the normal and cardiac denervated dog even after β-receptor blockade (Fig. 6).

Myocardial oxygen consumption was determined in seventeen instances before and with drug infusion. There was no consistent pattern or statistically significant change in myocardial oxygen consumption after administration of the drug. The mean values for these determinations were: 8.86 ± 0.72 ml./min before and 9.00 ± 0.66 ml./min following drug administration (Fig. 7).

Effects on peripheral vascular resistance

When Compound MJ-1988 was added to the venous reservoir while on total cardiopulmonary bypass there was a consistent drop in aortic pressure (Fig. 8). The drop in pressure occurred while the pump output was constant, so it is interpreted as a decrease in peripheral vascular resistance. The change in pressure occurred after α-receptor blockade with phenoxybenzamine and after β-receptor blockade with MJ-1999.

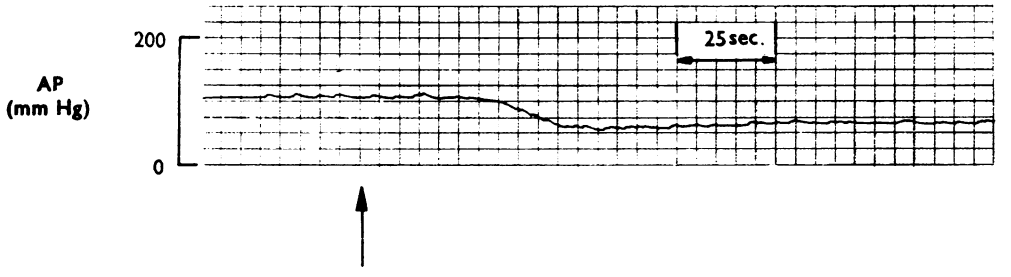

Fig. 8. Effect of infusion of Compound MJ-1988 on mean aortic pressure (MAP) with the output from the pump (systemic blood flow) held constant. The fall in pressure reflects a decrease in peripheral vascular resistance.

DISCUSSION

The results of these experiments indicate that Compound MJ-1988 produces an increase in myocardial contractility. In most experiments, the changes in all of the indices of contractility were indicative of a positive inotropic effect; at least two of the indices gave evidence of a positive effect in every case. Maximum dp/dt increased significantly in every instance, suggesting an augmentation of the velocity of contraction. The increase in maximum dp/dt occurred in some instances during a fall in left ventricular end-diastolic pressure. This is further evidence of a potent effect of the drug, and not of changes in mechanical determinants of maximum dp/dt, since a fall in left ventricular end-diastolic pressure of itself should result in a fall in maximum dp/dt (Wallace, Skinner & Mitchell, 1963).

MJ-1988 produced a left shift of the ventricular function curve only when the control curve showed depressed function. The inability to show a left shift of a function curve, when control function is not depressed (high stroke work from a relatively low end-diastolic pressure) is not surprising. Digitalis does not always influence such a curve in a positive manner.

The ability to show a positive inotropic effect in the cardiac denervated dogs and following β-receptor blockade in both the normal and the cardiac denervated dogs indicates that Compound MJ-1988 has a direct effect on cardiac muscle cells and is not dependent on activation of catecholamine receptors, release of stored catecholamines, or on activation of cardiac autonomic reflexes.

It would appear that the significant increase in coronary blood flow was the result of a direct coronary vasodilatation since it occurred at a constant mean aortic pressure in both the normal dog and in the cardiac denervated animal, and following β-receptor blockade. The peripheral vasodilatation seen after MJ-1988 also appeared to be a direct effect on the peripheral vessels since it occurred following α- and β-receptor blockade.

Of particular interest is the observation that the apparent increase in contractility was not associated with an increase in myocardial oxygen consumption. One might have anticipated an increase in oxygen utilization in view of the consistent increase in maximum dp/dt. Velocity of contraction has been shown to be an important determinant of myocardial oxygen consumption (Sonnenblick, Ross, Covell, Kaiser & Braunwald, 1965). The explanation may lie in a fortuitous decrease in mural tension as velocity of contraction was increased, since developed tension is also an important determinant of myocardial oxygen consumption. Nevertheless, from the standpoint of its therapeutic potential, the absence of an increase in oxygen requirement during the pharmacodynamic effects is encouraging. It is also noteworthy that peripheral vasconstriction is not an associated effect, nor is an increase in cardiac afterload.

Further and intelligent development of this drug requires rationalization of its application. There are certain clinical situations in which the demonstrated pharmacodynamic actions would be useful. The patient with congestive heart failure who is sensitive to, or refractory to, cardiac glycosides might be benefited. When interim cardiac support besides digitalis is indicated, as in certain cases of "shock," such an agent might be more beneficial than adrenergic drugs which exhibit α- and β-receptor stimulating properties, such as noradrenaline. The combination of myocardial support and peripheral vasodilatation is particularly appealing in this regard. The agent may also find use as a substitute for indirect acting sympathomimetic amines, such as mephentermine, in those patients with depleted myocardial catecholamines (Chidsey, Braunwald & Morrow, 1965). In this latter regard MJ-1988 is not subject to inactivation in the acidotic state which is a problem with the catecholamines (Aviado, 1965). The prominent pulmonary vasodilator actions of MJ-1988 (Aviado, Folle & Pisanty, 1967) is pertinent to the above suggested usages and would be particularly important in cor pulmonale and certain cases of shock. Finally, the coronary vasodilating properties of MJ-1988 suggest interesting possibilities which may be further explored. These therapeutic potentialities presuppose that the agent could be shown to have the same pharmacological properties in man at doses which were safe and free of limiting side effects. Further investigation is warranted.

SUMMARY

1. Compound MJ-1988 (6,7-dimethoxy-4-ethylquinazoline) was infused into normal and cardiac denervated dogs, at the rate of 0.375 mg/kg/min, while on a right heart bypass which permitted control of heart rate, cardiac input and arterial blood pressure.

2. MJ-1988 produced a positive inotropic effect as evidenced by a fall in LVEDP, increase in max dp/dt and a shortening of the duration of systole. Coronary blood flow increased, but myocardial oxygen consumption was unaffected. Peripheral vascular resistance was diminished by the agent.

3. The pharmacological effects were present in both normal and cardiac denervated dogs and after adrenergic receptor blockade.

4. A cardiotropic drug with the above actions might have clinical application and merits further study.

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REFERENCES

AVIADO, D. M. (1965). Pharmacologic approach to the treatment of shock. *Ann. intern. Med.*, **62**, 1050–1059.

AVIADO, D. M., FOLLE, L. E. & PISANTY, J. (1967). The cardiopulmonary effects of a quinazoline (MJ 1988): cardiac stimulant, pulmonary vasodilator and bronchodilator. *J. Pharmac. exp. Ther.*, **155**, 76–83.

CHIDSEY, C. A., BRAUNWALD, E. & MORROW, A. G. (1965). Catecholamine excretion and cardiac stores, of norepinephrine in congestive heart failure. *Am. J. Med.*, **39**, 442–451.

COOPER, T., GILBERT, J. W., BLOODWELL, R. D. & CROUT, J. R. (1961). Chronic extrinsic cardiac denervation by regional neural ablation. Description of the operation, verification of the denervation, and its effects on myocardial catecholamines. *Circulation Res.*, **9**, 275–281.

COOPER, T., WILLMAN, V. L., JELLINEK, M. & HANLON, C. R. (1962). Heart autotransplantation: Effect on myocardial catecholamines and histamine. *Science, N.Y.*, **138**, 40–41.

LISH, P. M., COX, R. H., DUNGAN, K. W. & ROBBINS, S. I. (1964). Some non-adrenergic bronchodilators and cardiac stimulating agents. *Pharmacologist*, **6**, 181.

LISH, P. M., WEIKEL, J. H. & DUNGAN, K. W. (1965). Pharmacological and toxicological properties of two new β-adrenergic receptor antagonists. *J. Pharmac. exp. Ther.*, **149**, 161–173.

SARNOFF, S. J. & BERGLUND, E. (1954). Ventricular function. 1. Starling's law of the heart studied by means of simultaneous right and left ventricular function curves in the dog. *Circulation*, **9**, 706–718.

SONNENBLICK, E. H., ROSS, J., JR., COVELL, J. W., KAISER, G. A. & BRAUNWALD, E. (1965). Velocity of contraction as a determinant of myocardial oxygen consumption. *Am. J. Physiol.*, **209**, 919–927.

WALLACE, A. G., MITCHELL, J. H., SKINNER, N. S. & SARNOFF, S. J. (1963). Duration of the phases of left ventricular systole. *Circulation Res.*, **12**, 611–619.

WALLACE, A. G., SKINNER, N. S., JR. & MITCHELL, J. H. (1963). Hemodynamic determinants of the maximal rate of rise of left ventricular pressure. *Am. J. Physiol.*, **205**, 30–36.

WILLMAN, V. L., COOPER, T., CIAN, L. G. & HANLON, C. R. (1962). Autotransplantation of the canine heart. *Surgery, Gynec. Obstet.*, **115**, 299–302.