Cardiovascular Effects of dl-1-(2-Acetyl-4-butyramidophenoxy)-2-hydroxy-3-isopropylaminopropane (M & B 17,803A), a New Cardioselective Beta Adrenoreceptor Blocking Agent*

Atul R. Laddu, M.B.B.S., M.D.**

SUMMARY

The direct and beta adrenergic blocking effect of M & B 17,803A was investigated in the isolated supported dog heart preparation (ISHP) in the presence of a constant coronary blood flow. Administration of graded doses of norepinephrine into the coronary arteries produced a dose-dependent increase in the heart rate, myocardial contractile force, left ventricular systolic pressure and coronary artery perfusion pressure. The myocardial oxygen consumption was increased significantly (p<0.05) by each dose of norepinephrine. Pretreatment of the ISHP with 0.3 mg/Kg of M & B 17,803A produced a generalized myocardial depression and an increase in coronary artery perfusion pressure, whereas the smaller dose (0.15 mg/Kg) of the blocking agent was without any effect. Both the doses of M & B 17,803A produced a competitive blockade of the effect of norepinephrine on the myocardial hemodynamics and oxygen consumption. M & B 17,803A thus appears to be a potent agent that blocks the beta receptors, and has a significant myocardial depressant activity.

Additional Indexing Words:
Beta adrenergic blocking agents   Cardioselective beta receptor blockade
Coronary vascular resistance       Myocardial oxygen consumption
Norepinephrine

During the past few years, several newer beta adrenoreceptor blocking agents have been synthesized.11,13,25,35,37 The major disadvantages of these agents include a cardiac depression10,13,21,44 and a lack of selectivity of action. Recently, a new beta adrenergic blocking agent, dl-1-(2-acetyl-4-butyramidophenoxy)-2-hydroxy-3-isopropylaminopropane (M & B 17,803A) has been synthesized47 in an attempt to produce a cardioselective beta receptor blocking action with a minimal myocardial depressant action. The present

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* Generic name of M & B 17,803A (IL 17,803A) is acebutolol.
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investigation is concerned with the direct and beta adrenoreceptor blocking actions of M & B 17,803A in the isolated supported dog heart preparation (ISHP) under controlled hemodynamic conditions. The structure of M & B 17,803A is shown in Fig. 1.

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\text{Fig. 1. Structure of M & B 17,803A.}
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**METHODS**

The isolated supported dog heart preparation

The present study was conducted in the ISHP with a constant coronary blood flow. The details of the technique have been reported in several previous communications. Briefly, the heart of a recipient dog was isolated. Heparinized, oxygenated arterial blood, maintained at 37° C in a reservoir, obtained from a donor dog, was pumped through both the coronary arteries. The blood in the reservoir was stirred constantly to insure proper mixing. The coronary blood flow was adjusted at the beginning of the experiment to provide a coronary artery perfusion pressure of 100 mmHg, and the flow was then kept constant throughout the experiment. Since the descending aorta is tied, the blood flows through both the coronary arteries and drains itself into the right ventricle. The oxygen tension of the arterial (pAO2) and the venous (pVO2) blood was recorded continuously on a Grass polygraph. For recording the myocardial contractile force, a Brodie-Walton strain gauge arch was sutured to the left ventricle. The left ventricular peak systolic pressure was measured by inserting a thin latex balloon into the left ventricular cavity through an opening in the left atrium and inflating it with 1-5 ml of saline. The total coronary venous drainage, less a small amount of Thebesian drainage, was returned to the cannulated femoral vein of the donor dog via a cannula in the right ventricle. Heart rate (ECG) was recorded by inserting the leads in the retracted chest wall, corresponding to standard lead II. The myocardial oxygen consumption (MVO2) expressed in ml/100 Gm heart wt/min was calculated from the arteriovenous oxygen difference, the total coronary blood flow, the heart weight and hemoglobin level in the blood according to the method described before. Experimental design

The ISHP was allowed to stabilize for 15–30 min. Norepinephrine was administered into the coronary arteries near the brachiocephalic cannula in doses ranging from 0.06 to 2.0 μg at 10 min intervals. M & B 17,803A was then infused slowly into the femoral vein of the donor dog in a dose of 0.15 and 0.30 mg/Kg and norepinephrine repeated again over a dose range of 1.0 to 32.0 μg intraarterially, 30 min after the administration of M & B 17,803A.

The following drugs were used: norepinephrine bitartarate (Sterling-Winthrop Research Institute, Rensselaer, NY) and M & B 17,803A. Both the drugs were dissolved in normal saline solution.
RESULTS

Isolated supported dog heart preparation

We have observed that the ISHP is a very stable preparation and its hemodynamic functions are very well maintained over a period of 6 hours.21),24) There is no hemolysis or ventricular ectopic beats in most of the experiments, and experiments with either of these criteria are discarded. The ISHP differs from similar preparations described by Alanis et al2) and Sarnoff et al38) in 2 respects; first, the recipient heart was left in situ and second, the coronary arteries were perfused with oxygenated arterial blood at a constant flow rate.

The pH of the arterial blood remained fairly constant over the entire experimental period and ranged from 7.20 to 7.43 in the various experiments. Administration of either norepinephrine or M & B 17,803A did not have any significant effect on the arterial pH.

The hemoglobin level is an important determinant of the myocardial oxygen consumption and showed a fair degree of consistency, since it ranged from 14.0 to 14.6 Gm% in the various experiments (14.6 Gm% = 100%). Neither the agonist nor the antagonist produced any significant effect on the hemoglobin level in any of the ISHP’s.

The technique of using polarographic oxygen macroelectrodes to measure the arterial and the venous oxygen tension has been used extensively by us.21),24) We have shown earlier that the Beckman oxygen electrodes are more sensitive than the Guyton’s oxygen analyzer.21) Moreover, a continuous recording of the oxygen tension enables us to accurately detect the changes taking place in pO₂ at anytime. Because of the accuracy of the results and simplicity of

<table>
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<tr>
<th>Exp. No.</th>
<th>Heart rate (beats/min)</th>
<th>Myocardial contractile force (mm)</th>
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<td></td>
<td>Before</td>
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<td>1</td>
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<td>± SEM</td>
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$p < 0.05$

*p value was calculated by paired analysis.*
use, we prefer the use of the oxygen electrodes to any other technique of measuring oxygen tension.

The total coronary blood flow varied between 60 and 90 ml/100 Gm heart wt/min in the present series, and the various recipient hearts weighed between 120 and 180 Gm. A total of 6 successful ISHP’s was performed.

**Direct effects of M & B 17,803A in the ISHP**

We have observed that if the blocking agent is administered to the recipient heart, it is washed out quickly from the circulation and its high concentration might depress the myocardium suddenly. M & B 17,803A was, therefore, administered by a slow intravenous infusion over a period of 5 min to the donor dog. The smaller dose (0.15 mg/Kg) did not produce any significant effect on either the heart rate, myocardial contractile force, left ventricular peak systolic pressure, coronary artery perfusion pressure or the myocardial oxygen consumption. However, the larger dose (0.30 mg/Kg) consistently produced a reduction in the heart rate, myocardial contractile force and left ventricular systolic pressure approximately 10–15 min after the administration of the drug. The left ventricular end-diastolic pressure was unchanged by either dose of M & B 17,803A. The coronary artery perfusion pressure showed an upward trend, whereas there was no effect on the venous pO₂ (Table I). The effect of 0.30 mg/Kg of M & B 17,803A on myocardial hemodynamics lasted throughout the experimental procedure (3 hours), although the contractile force and left ventricular peak systolic pressure showed a 40% recovery at the end of 3 hours.

<table>
<thead>
<tr>
<th>17,803A (0.3 mg/Kg) in the ISHP</th>
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<td><strong>Left ventricular systolic pressure (mmHg)</strong></td>
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\( p < 0.05 \) \( p < 0.05 \) \( p > 0.1 \)
Adrenergic beta receptor blockade

When norepinephrine was injected into the coronary arteries, there was a dose-dependent increase in the heart rate, myocardial contractile force, left ventricular peak systolic pressure and coronary artery perfusion pressure. The myocardial oxygen consumption in the control experiments ranged from 5.59 to 5.70 ml/100 Gm heart wt/min (mean ± SEM=5.65 ±0.02 ml/100 Gm heart wt/min). The venous oxygen tension was reduced significantly following even the smallest dose of norepinephrine (0.06 µg). Such an effect in the presence of a constant coronary blood flow is indicative of an increased myocardial oxygen consumption. The myocardial oxygen consumption increased significantly in a dose-dependent manner after each injection of norepinephrine.

Pretreatment of the ISHP with either dose of M & B 17,803A produced an effective blockade of the positive inotropic and chronotropic effects of norepinephrine, since there was a parallel shift of the dose response curve of the agonist to the right (Fig. 2-4).

The effect of norepinephrine upon the coronary artery perfusion pres-

![Graph showing dose-response curve of norepinephrine on the heart rate before and after 2 doses of M & B 17,803A in the ISHP (N=6). Note the parallel shift in the curves indicating a competitive antagonism of the norepinephrine effect.](image-url)
Fig. 3. Blockade of the effect of norepinephrine on the myocardial contractile force (CF: as measured by the Brodie-Walton strain gauge arch) by M & B 17,803A in the ISHP (N=6).

sure was also blocked in a competitive manner as indicated by a parallel shift of the dose response curve to the right (Fig. 5). The dose-related increases in MVO₂ following various doses of the catecholamine were blocked by M & B 17,803A (Fig. 6).

**DISCUSSION**

M & B 17,803A has been reported to be a potent agent that blocks the adrenergic beta receptors. Basil et al studied the pharmacological properties of M & B 17,803A and observed that it differs from propranolol in being 1/6 as potent and possesses a cardioselectivity of action. M & B 17,803A is 20 times more potent than practolol in increasing the refractory period of isolated rabbit atria in vitro, and has local anesthetic and quinidine-like properties. This agent prevents the epinephrine-induced ventricular tachycardia and increases the dose of ouabain needed to produce cardiac arrhythmia in the dog heart-lung preparation. In the present series of investigations, M & B 17,803A in doses ranging from 0.15 to 0.30 mg/Kg produced a parallel shift of the dose-response curves of norepinephrine to the right,
indicating a competitive antagonism. We have shown earlier\textsuperscript{21,40} that both practolol and LB-46 produce a competitive blockade of the effect of isoproterenol upon the myocardial hemodynamics and oxygen consumption in the ISHP. The results are thus in agreement with those of Baird and Linnell\textsuperscript{4)} and Basil et al\textsuperscript{5)} who also showed a competitive blockade by M & B 17,803A. One interesting observation which needs to be mentioned here is that M & B 17,803A blocked all the effects of norepinephrine upon the myocardial hemodynamics and oxygen consumption in the ISHP to an equal extent. M & B 17,803A resembles practolol and LB-46 in that it blocks both the $\beta_1$ and $\beta_2$ receptors.\textsuperscript{21,40)}

The coronary artery perfusion pressure was increased following M & B 17,803A (Table I), indicating that the coronary vascular resistance was increased. M & B 17,803A thus resembles propranolol, pronethalol, MJ 1999 and INPEA\textsuperscript{10,16,26–29,31,43,46} which produce an increase in the coronary vascular resistance and a decrease in the coronary blood flow. It is generally thought that the decrease in the coronary blood flow observed after the beta receptor blocking agents is due to either a blockade of the beta receptors in the coronary arteries or to a decrease in the oxygen requirements of the heart.
Fig. 5. Blockade of the effect of norepinephrine on the coronary artery perfusion pressure (CAPP) by M & B 17,803A in the ISHP.

or both.\textsuperscript{21} We have reported earlier that practolol and LB-46 failed to increase the coronary artery resistance in the ISHP although they decreased the oxygen extraction by the heart.\textsuperscript{21,40} The data obtained with M & B 17,803A in which there was an increase in the coronary vascular resistance without any change in the oxygen consumption by the myocardium further emphasize the hypothesis that the changes in the coronary artery flow or resistance are not necessarily associated with the inhibition of the beta receptors in the heart or the coronary vessels.\textsuperscript{15,21}

M & B 17,803A, like propranolol, possessed a significant myocardial depressant activity in the ISHP,\textsuperscript{41} especially with the higher dose (0.3 mg/Kg). Amongst the various beta adrenoreceptor blocking agents tested in the ISHP, practolol, d-propranolol, MJ 1999 and LB-46 possessed no myocardial depressant activity.\textsuperscript{21,23,39,41} Basil and coworkers\textsuperscript{6} observed that M & B 17,803A produces less myocardial depression than propranolol in the anesthetized dog. Our results are not in agreement with those of Cuthbert and Owusu-Ankomah\textsuperscript{8,9} who did not observe any change in the heart rate and systolic or diastolic pressure following oral doses of up to 300 mg of M & B 17,803 A in healthy human volunteers. The reason for this discrepancy is not very clear,
Fig. 6. Blockade of the effect of norepinephrine on the myocardial oxygen consumption (MVO₂) in the ISHP.

but may be explained on the basis of the difference in the experimental design.

Most of the beta adrenergic blocking agents tested produce a blockade of all the beta receptors.¹²,¹³,²⁵ The first "cardioselective" beta adrenoceptor blocking agent developed was practolol.¹¹ The cardioselective blocking agents are of great importance in the therapy of cardiac diseases.⁴ The structural requirements for the cardioselectivity have been reported by Ablad et al,¹ Bagwell and Vaughan-Williams³ and Vaughan-Williams et al.⁴⁵ It has been postulated that substitution of the side chain at para position of the benzene ring yields cardioselectivity, whereas either ortho or meta substitution results in a loss of such an activity. Thus, the para substituted analogs of oxprenolol and alprenolol showed cardioselectivity whereas the ortho analog of practolol had no cardioselectivity.¹¹,⁴⁵ Further, the ortho analog of 1-t-butylamino-3-(methoxyphenoxy)-2-propanol (M66527) was 3 to 4 times more potent on peripheral beta receptors and the para analog (M66368) was 2 to 4 times more potent on the cardiac beta receptors.³ It is interesting to note that M & B 17,803A has a side chain attached at the para position, which may explain the cardioselective nature as reported by several workers. However, there seems to be a discrepancy as to whether M & B 17,803A is
cardioselective in its action or not. Basil et al,5) Khambatta17) and Roetscher36) are of the opinion that M & B 17,803A selectively blocks the cardiac beta receptors. On the other hand, Briant et al7) and George et al14) feel to the contrary. Cuthbert and Owusu-Ankomah8) failed to show any selectivity of action using heart rate and fall in the diastolic blood pressure following intravenous isoproterenol. Baird and Linnell4) observed a moderate degree of cardioselectivity with M & B 17,803A.

We have shown earlier20),42) that substitution of a methyl group in the 3 position of the benzene ring yielded a potent beta receptor blocking agent (Kö 592) which had a pA₂ value of 8.3 compared to the 2-methyl derivative (Kö 589; pA₂ 6.8) and the 4-methyl derivative (Kö 612; pA₂ 6.9). The 3-methyl derivative also possessed more local anesthetic activity and could effectively antagonize epinephrine-induced and ouabain-induced cardiac arrhythmias.42) In some preliminary experiments, it appears that the 4-methyl compound (Kö 612) is relatively cardioselective (Laddu, unpublished observations). Further experiments are in progress to study whether Kö 612 does possess cardioselectivity in experimental animals. It should therefore be noted that the potency of an agent to block the beta receptors in the body does not necessarily parallel the ability to block cardiac beta receptors in a selective manner.

In recent years, several methods have been suggested to assess the potency and cardioselectivity of the beta adrenoreceptor blocking agents.4) Such screening procedures include in vitro guinea pig preparations of cardiac muscle and tracheal smooth muscle,1) dog or cat heart rate in vivo and guinea pig tracheal smooth muscle in vitro,11) dog heart rate and perfused hind limb in vivo5),35) and guinea pig heart rate and anaphylactic bronchospasm in vivo.5) The ISHP is another valuable preparation which is very helpful in screening such drugs since one can study the effect of beta receptor agonists and antagonists on the cardiac and coronary beta receptors.

We have reported earlier that agents such as practolol and LB-46 when administered intravenously to the donor dog in the ISHP produce a decrease in the myocardial oxygen consumption as observed by an increase in the venous oxygen tension.21),24),41) This effect has been referred to as the “oxygen sparing effect”.19) The oxygen sparing effect does not appear to be related to a blockade of beta receptors since Sch 11973 [N-2(1-phenylisopropyl)-N'-p-toluene sulfonyl urea] and nitroglycerin which produce a similar effect in the ISHP do not block beta receptors.19),21) The exact mechanism of the oxygen sparing effect of these agents is not yet clearly known, but several investigators believe it to be due to a shift of the oxygen-dissociation curve of hemoglobin to the right.30),33) In the ISHP, M & B 17,803A did not produce any effect...
on the myocardial oxygen consumption in the doses used. It might be interesting to study the effect of higher doses of M & B 17,803A in the ISHP, since it has been shown that propranolol also is probably effective in a higher dose.\textsuperscript{33) However, one cannot rely too much on the possible effect observed with a larger dose of M & B 17,803A upon the myocardial oxygen consumption, since even the 0.3 mg/Kg dose produced a significant decrease in the myocardial hemodynamics in the ISHP, and a still higher dose might have a greater depressant effect.

In our previous studies concerning the effects of catecholamines and adrenergic blocking agents upon the nutritional circulation in the ISHP, we have observed that vasodilators such as isoproterenol produce a reduction of the clearance of \textsuperscript{86}Rubidium.\textsuperscript{41) On the other hand, norepinephrine increased the coronary artery perfusion pressure and still produced a reduction in the capillary blood flow in the heart.\textsuperscript{23) Practolol and LB-46 which are potent beta adrenergic blocking agents and have no effect upon the coronary vascular resistance in the ISHP, produce an increase in the clearance of \textsuperscript{86}Rubidium and the capillary transport coefficient, PS.\textsuperscript{23,41}) It will be interesting to study the effect of M & B 17,803A on the capillary blood flow in the ISHP.

Beta adrenergic blocking agents have been used successfully in the treatment of angina pectoris.\textsuperscript{12,21,32,34) Although propranolol has been used extensively in the clinical management of angina, its main disadvantage is the production of a direct myocardial depression. M & B 17,803A has been reported to be effective in the treatment of angina pectoris,\textsuperscript{17) although it also possesses myocardial depressant activity in the ISHP. It therefore appears that one needs to develop a beta receptor blocking agent that will have either a minimal or no depressant action on the heart and will still be useful in angina pectoris.

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