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# Effects of a Novel Vesamicol Receptor Ligand, *m*-(Iodobenzyl)trozamicol, on the Canine Isolated, Blood-Perfused Atrioventricular Node Preparation

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ABSTRACT—*m*-(Iodobenzyl)trozamicol (MIBT) is a recently discovered vesamicol analogue. It has been shown that radiolabelled [$^{125}$I]MIBT can be used as a marker of cholinergic innervation in the heart as well as in the brain. The purpose of this study was to analyze the direct effects of MIBT on the atrioventricular and intraventricular conduction in addition to the coronary blood flow using the canine isolated, blood-perfused atrioventricular node preparation. Intracoronary administration of MIBT suppressed the atrioventricular and intraventricular conduction, while it increased the coronary blood flow. The effect and duration of action on the intraventricular conduction was less pronounced compared with other effects. Moreover, the doses of MIBT needed to cause negative dromotropic and coronary vasodilator effects in this study was much greater than those needed for imaging the cardiac cholinergic innervation. Pretreatment of the preparations with a muscarinic receptor antagonist, atropine, did not block these effects of MIBT, suggesting that MIBT may possess muscarinic receptor-independent ion channel activity in the cardiac conduction system and coronary arteries.

*Keywords*: *m*-(Iodobenzyl)trozamicol (MIBT), Vesamicol, AV node, Conduction, Coronary blood flow

In the cholinergic nerve terminals, acetylcholine is transported from the cytoplasm into the vesicle by a specific transporter that can be blocked by vesamicol (1 – 4). MIBT, *m*-(iodobenzyl)trozamicol, is a recently discovered vesamicol analogue that can selectively bind to vesamicol receptors in vivo much like vesamicol itself (1 – 4). Recent animal studies demonstrated that radiolabelled [$^{125}$I]MIBT can be used as a marker of cholinergic function in the brain (1 – 3). More recently, it has been shown that MIBT can be used to probe the cholinergic innervation in the conduction system in the *in situ* heart, which will make it possible to study disease processes including aging and ischemia together with the effects of drugs and the catheter ablation procedure (4). However, direct as well as indirect effects of MIBT on the cardiac conduction system are still lacking.

The purpose of the present study was to analyze the potential direct effects of MIBT on the atrioventricular and intraventricular conduction and the coronary blood flow using the canine isolated, blood-perfused atrioventricular node preparation (5 – 8), since such knowledge is of potential clinical relevance. In addition, after the direct effects of MIBT were assessed, the preparation was treated with a muscarinic receptor antagonist atropine to study the mechanism of the action of MIBT on the cardiac conduction and coronary blood flow (9).

## MATERIALS AND METHODS

All experiments were performed in accordance with the Guidelines for Animal Experiments, Yamanashi Medical University. Experiments were carried out using the atrioventricular node preparation cross-circulated with heparinized arterial blood of a donor dog as previously described (5 – 8).

### *The canine isolated, blood-perfused AV node preparation*

*Isolated preparations*: The isolated atrioventricular node preparations were obtained from beagle dogs of either sex, weighing about 10 kg. The dog was anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and given calcium

heparin (500 U/kg, i.v.). The heart was excised after exsanguination and plunged into cold Tyrode's solution kept at 4°C. The atrioventricular node preparation consisted of both the right atrium and interventricular septum. The right coronary artery and the anterior septal artery were directly cannulated, while the atrioventricular node artery was cannulated through the left circumflex artery. Bipolar pacing electrodes were sutured onto the sinus nodal region, while bipolar recording electrodes were attached onto the right atrium (A), His bundle (H) and the base of the anterior papillary muscle of the right ventricle (V) (5 – 8).

*Blood-donor dogs*: Male beagle dogs weighing about 15 kg were used for the blood-donor dogs. The dog was anesthetized initially with sodium pentobarbital (30 mg/kg, i.v), and given calcium heparin (500 U/kg, i.v). Respiration was controlled using an animal ventilator (SN-480-3; Shinano, Tokyo). The systemic blood pressure, lead II electrocardiogram and heart rate were continuously monitored using a polygraph system (RM-6000; Nihon-Kohden, Tokyo). Arterial blood gases were kept within the physiological range by adjusting the respiratory rate and oxygen supplementation.

*Cross-circulation*: The preparation was placed in a double-wall glass jacket maintained at 38°C by circulating warm water and was perfused with arterial blood from the carotid artery of the donor dog. Perfusion pressure was kept at 120 mmHg with a peristaltic pump (7553-00; Cole-Parmer, Chicago, IL, USA) and Starling's pneumatic resistance placed parallel to the perfusion circuit. Venous blood from the preparation and excess blood passing through the pneumatic resistance were collected in a blood reservoir and returned to the jugular vein of the donor dog.

*Parameters measured*: The atrioventricular node preparation was electrically driven at a fixed rate of 150 beats/min by a stimulator (SEN-7203, Nihon-Kohden) and the isolation unit (SS-201J, Nihon-Kohden) with rectangular pulses of 1 – 3 V (about 50% above the threshold voltage) and 5-ms duration. The AH and HV intervals were measured individually with an analysis pitch of 1 ms using an automatic interval meter (DHM-226-1; Dia Medical, Tokyo). The coronary blood flow through each nutrient artery of the preparation was monitored using an electromagnetic flowmeter (MVF-1100, Nihon-Kohden). It has been shown that drugs injected into the atrioventricular nodal artery selectively affect the AH interval, while those injected into the anterior septal artery predominantly affect the HV interval (5 – 8).

*Experimental protocol*

Once the preparations were stabilized, MIBT in doses of 1.8 – 547.1 nmol (1 – 300 $\mu$g) or the respective vehicle solution was injected into either the atrioventricular node artery or the anterior septal artery using a small microsyringe (Ito, Tokyo) in a volume of 10 – 30 $\mu$l over 4 s. Physiological recordings were performed for 10 min after each dose. Because a relatively small amount of drug was administered to the preparation compared to that needed in a whole animal model, multiple drug doses were studied in the same preparation (5 – 10). The effluent blood through each preparation immediately after the drug injection was discarded to eliminate the drug effects on the blood-donor dog. Having assessed the effects of the drug and vehicle solution, a muscarinic receptor antagonist, atropine in a dose of 7.4 nmol (5 $\mu$g), was administered to the preparation. Then, MIBT in doses of 1.8 – 547.1 nmol was administered to compare the effects of MIBT on each parameter with those recorded before atropine treatment.

*Drugs*

MIBT, (*dl*)-*m*-(iodobenzyl)trozamicol dihydrochloride (MW = 548.32), was generously provided by Radiopharmaceutical Development Group, Department of Radiology, University of Minnesota, while the following drugs were purchased: pentobarbital sodium (Tokyo-Kasei, Tokyo), heparin calcium (Mitsui, Tokyo), acetylcholine chloride (Dai-ichi, Tokyo ) and atropine sulfate (Tanabe, Osaka). MIBT was dissolved in 25% ethanol-water in a concentration of 10 mg/ml and then diluted with saline to prepare the 1 mg/ml and 100 $\mu$g/ml solutions.

*Data analysis and statistics*

Peak responses in each parameter are expressed as a percent of their basal values before the drug injection. The pharmacodynamics of the drug was assessed using the onset half time (OHT) and recovery half time (RHT), as defined previously (7, 10). Briefly, the OHT is the time (s) required to reach the peak from the 1/2 peak effect after the injection of the highest dose of MIBT. $OHT_{(AH)}$, $OHT_{(HV)}$ and $OHT_{(CBF)}$ indicate the onset half times to the maximum responses in the AH and HV intervals and coronary blood flow, respectively. Meanwhile, the RHT is the time (s) required to return to 1/2 peak effect from the peak after the injection of the highest dose of MIBT. $RHT_{(AH)}$, $RHT_{(HV)}$ and $RHT_{(CBF)}$ indicate the recovery half times from the maximum responses in the AH and HV intervals and coronary blood flow, respectively. The data are presented as the mean ± S.E.M. The statistical comparisons of mean values within a group were carried out using one-way repeated-measures ANOVA followed by Contrast, while those between the drug and vehicle were evaluated by two-way repeated-measures ANOVA followed by the *t*-test for unpaired data. A P-value less than 0.05 was considered significant.

## RESULTS

The basal AH interval before the drug injection was 115 ± 4 ms (n = 4). MIBT injected into the atrioventricular node artery increased the AH interval in a dose-related manner as shown in Figs. 1A and 2A, while the HV interval was hardly affected (not shown). The maximum change after the highest dose of MIBT was +34.7 ± 2.5%, while $OHT_{(AH)}$ and $RHT_{(AH)}$ values were 13 ± 3 s and 38 ± 9 s, respectively. The complete atrioventricular conduction block was observed transiently after the injection of the highest dose of MIBT in 1 preparation out of 4.

The basal HV interval before the drug injection was 40 ± 1 ms (n = 4). MIBT injected into the anterior septal artery increased the HV interval in a dose-related manner as shown in Figs. 1B and 2B, while the AH interval was hard-

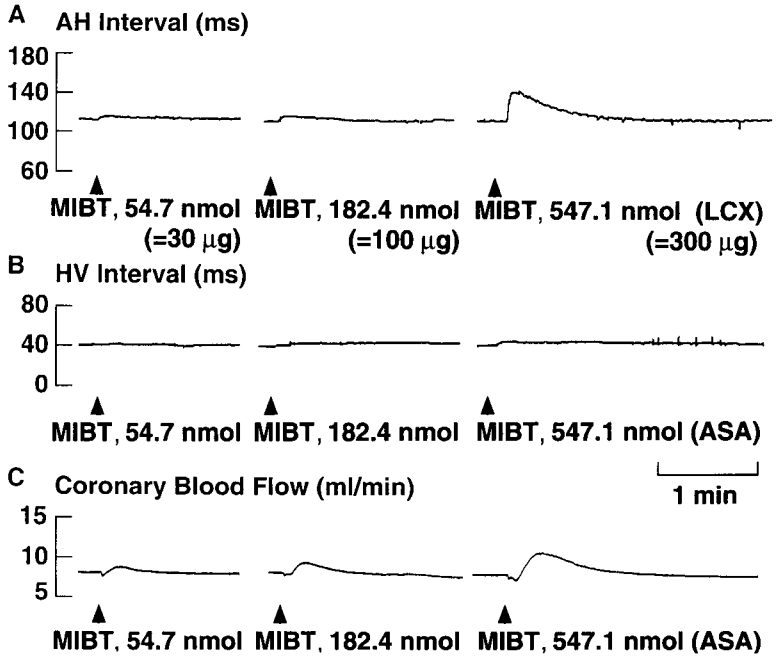


**Fig. 1.** Original tracings of the effects of MIBT on the AH interval (A), HV interval (B) and coronary blood flow through the anterior septal artery (C). MIBT injected into the atrioventricular node artery via the left circumflex artery (LCX) selectively affects the AH interval (A), while that injected into the anterior septal artery (ASA) predominantly affects the HV interval (B).

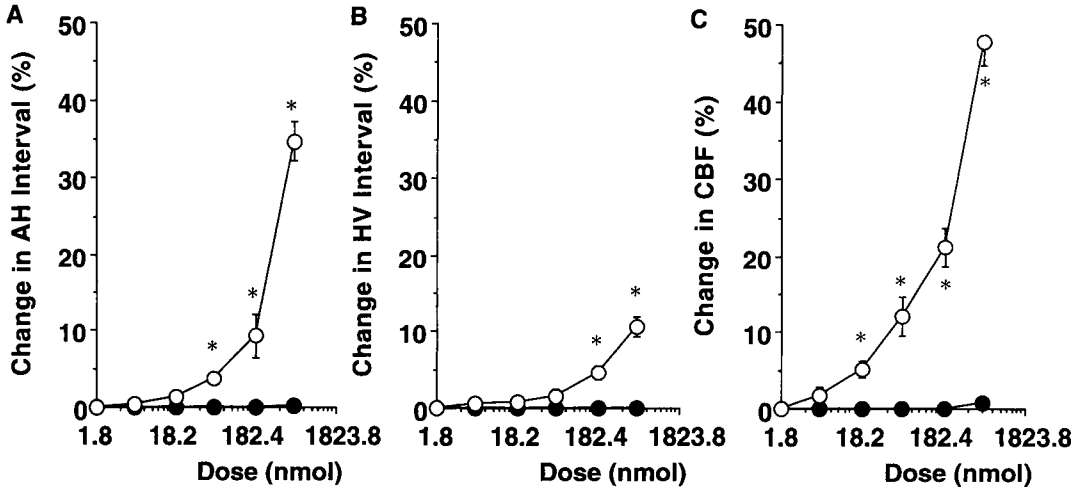


**Fig. 2.** Dose-response curves of the effects of MIBT (open circles) and its vehicle solution (closed circles) on the AH interval (A), HV interval (B) and coronary blood flow (CBF) (C). The data are presented as the mean ± S.E.M. (n = 4). *P<0.05, vs respective results by vehicle solution.

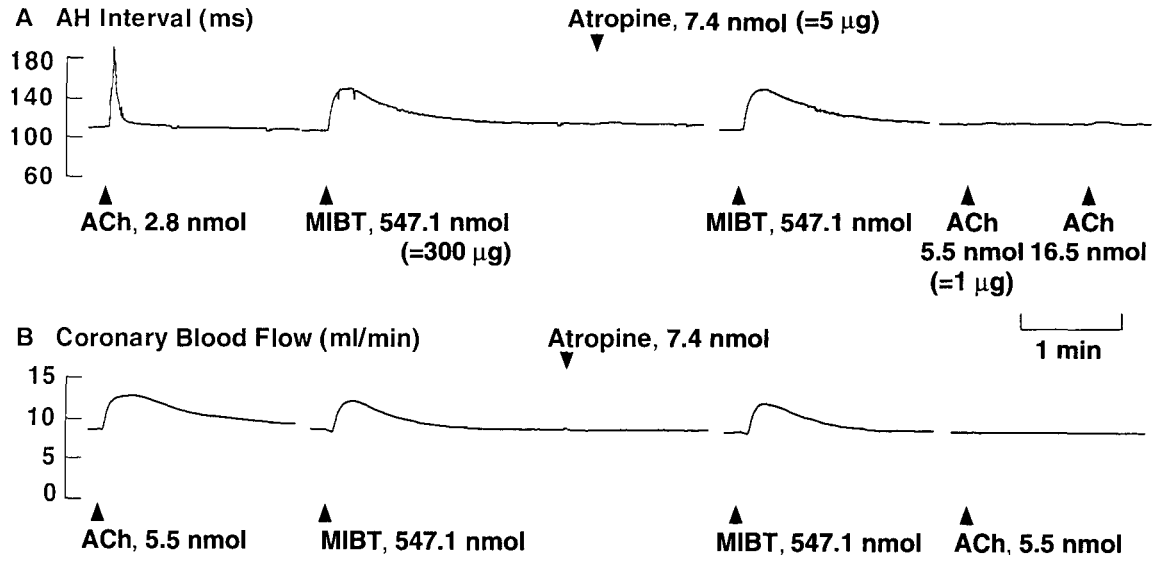


**Fig. 3.** Typical tracing of the AH interval (A) and coronary blood flow through the anterior septal artery (B) showing the effect of atropine on the MIBT-induced changes in each parameter. While the negative dromotropic effect and coronary vasodilator action by acetylcholine (ACh) were significantly attenuated, MIBT exerted the same extent of changes in each parameter as those observed before atropine treatment.

ly affected (not shown). The maximum change after the highest dose of MIBT was $+10.6 \pm 1.3\%$, while $OHT_{(HV)}$ and $RHT_{(HV)}$ values were $4 \pm 1$ s and $13 \pm 1$ s, respectively.

The basal coronary blood flow through the anterior septal artery before the drug injection was $7.8 \pm 1.0$ ml/min ($n=4$). MIBT increased the blood flow in a dose-related manner as shown in Figs. 1C and 2C. The maximum change after the highest dose of MIBT was $+47.8 \pm 3.1\%$, while $OHT_{(CBF)}$ and $RHT_{(CBF)}$ values were $9 \pm 1$ s and $20 \pm 2$ s, respectively.

The administration of atropine in a dose of 7.4 nmol, which effectively blocks the muscarinic receptor at least for 30 min in this model (9), significantly attenuated effects of acetylcholine, but it did not affect the negative dromotropic or coronary vasodilator effects of MIBT in doses of 1.8 – 547.1 nmol ($n=4$). Typical experiments demonstrating the lack of blocking effect of atropine on the highest dose of MIBT-induced changes are shown in Fig. 3.

## DISCUSSION

The radiolabelled [$^{125}$I]MIBT has been recently shown to be a useful quantitative marker for assessing the cholinergic innervation in the cardiac conduction system (4). Since the information regarding potential effects of MIBT on the cardiac conduction system is still lacking, we assessed its direct effects on the AH and HV intervals in addition to the coronary blood flow using the well-established canine isolated, blood-perfused atrioventricular node preparation (5 – 8). As demonstrated in the present study, MIBT suppressed the atrioventricular conduction and increased the coronary blood flow, while the inhibitory effect on the intraventricular conduction, as determined by measuring the effect of MIBT on His-ventricular conduction, was less potent and shorter-acting than the other effects. This is the first report describing the negative dromotropic effect of MIBT.

MIBT has been reported to exert an anticholinergic effect via a potent inhibition of the vesicular acetylcholine transport system like vesamicol in the in vivo model (1 – 3). One observation of this study is that MIBT administration resulted in the negative dromotropic and coronary vasodilator effects that is expected for cholinomimetic drugs, but atropine did not attenuate these effects of MIBT. This suggests that a non-cholinergic pathway may be involved in the cardiovascular effects of MIBT. We have shown that both calcium and sodium channel blockers can increase AH interval and coronary blood flow, but the effects of calcium channel blockers were more potent and longer-lasting than those of sodium channel inhibitors (5 – 8, 10). Moreover in those studies, only sodium channel inhibitors prolonged the HV interval. Thus, the current results suggest that MIBT may possess muscarinic receptor-independent ion channel activity in the cardiac conduction system and coronary arteries.

Another important finding is the relation between the doses of MIBT causing the observed electrophysiological changes in this study and those of radiolabelled MIBT needed for imaging the cardiac cholinergic innervation. In our previous studies with several sodium and calcium chan-

nel blockers (5 – 8, 10), the extent of maximal changes in each parameter of the cross-circulated isolated preparation was similar between those obtained by direct administration of 10 $\mu$g of a drug to the nutrient coronary artery and those by intravenous administration of 100 – 300 $\mu$g/kg of the drug to the blood-donor dog. In recent work, the radioligand [$^{125}$I]-MIBT with a specific activity of 150 – 200 Ci/mmol was given to the rat at the doses of 5.0 – 6.7 nmol to quantitate regional differences of cholinergic innervation in the heart (4), which can be extrapolated to be 23.2 nmol/kg of currently used MIBT. Taken together, the current results suggest that the doses of the radiolabelled [$^{125}$I]-MIBT needed for assessing the cardiac cholinergic function might be at least ten times smaller than the doses of MIBT affecting the cardiac function in this study. In addition, for in vivo imaging, we can now use [$^{123}$I]MIBT instead of [$^{125}$I]MIBT. Since the specific activity of [$^{123}$I]MIBT could be as high as 25,000 Ci/mmol with a half-life of >13 h, the actual injected mass of the compound would be 9.1 – 18.2 nmol for a patient. As such, with this degree of safety, it may be possible to develop a relatively safe vesamicol receptor radioligand for imaging the cholinergic nerves in the heart.

In conclusion, MIBT possesses muscarinic receptor independent negative dromotropic and coronary vasodilator effects. Since the doses of MIBT needed for imaging the cardiac cholinergic function are calculated to be much smaller than those affecting the conduction system and coronary blood flow, MIBT can be used safely in future clinical applications to study a number of disease processes related to the structural and functional alterations of the parasympathetic nervous system.

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