Effects of Atenolol and Some Other Beta-Adrenoceptor Blocking Agents on Norepinephrine-Induced Responses in Isolated and Perfused Rat Hearts

Junko NAKASONE, Takayuki KATO, Katsuhiko NOGUCHI, Fumio NAGAMINE and Matao SAKANASHI

Department of Pharmacology, School of Medicine, Faculty of Medicine, University of the Ryukyus, Nishihara-cho, Okinawa 903-01, Japan

Accepted April 30, 1988

Abstract—The effects of atenolol and another three beta-adrenoceptor blocking agents on norepinephrine (NE)-induced cardiac responses were examined in isolated and perfused rat hearts following a Langendorff method. Bolus injection of atenolol did not show significant inhibitory effects on NE-induced increases in myocardial contractile force (MCF) and heart rate. Bolus injections of metoprolol and timolol were also ineffective for inhibiting NE-responses. However, both bolus injection and infusion of propranolol or infusion of atenolol, metoprolol and timolol all significantly inhibited NE-responses. On sustained increase in MCF induced by infusion of NE, the inhibitory effect of atenolol was transient, while that of propranolol was continuous. From these results, it is concluded that atenolol displays a different time course of action on NE-induced cardiac responses by bolus injection or infusion because of its pharmacological properties, which may be due to its low lipophilicity.

Atenolol has been clinically utilized for treatment of patients with hypertension (1-3) and angina pectoris (4). These clinical uses are based on basic research indicating that atenolol has a long-term hypotensive activity in vivo in spontaneously hypertensive rats (5, 6) and that the drug has a beta, -adrenoceptor blocking effect without intrinsic sympathomimetic and membrane stabilizing activities in the isolated guinea pig atrium (7, 8) and rat atrium (9), isolated rabbit papillary muscle (10), blood-perfused dog hind-limb preparation (11), and anesthetized dogs (10, 12, 13) and rats (5, 6) in vivo. However, it has not been established how atenolol acts on whole hearts, especially isolated whole hearts. Therefore, the present experiment was designed to investigate the detailed effects of atenolol on whole heart; and for this purpose, the actions of atenolol on cardiac responses to norepinephrine (NE) were examined in isolated rat hearts which were perfused following a Langendorff method, and they were compared with those of other beta-adrenoceptor blocking agents.

The results indicated that different routes of administration of beta-adrenoceptor blocking agents in these preparations showed different time courses for the inhibition of NE-responses.

Materials and Methods

Male Wistar rats weighing 250–350 g were killed by a blow on the neck, and the hearts were rapidly isolated. The aorta was cannulated, and the heart was perfused retrogradely via the aortic cannula according to the method of Langendorff without recirculation at a constant flow rate of 8–10 ml/min with modified Krebs-Henseleit solution containing: 120.0 mM NaCl, 4.8 mM KCl, 1.25 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25.0 mM NaHCO₃ and 11.0 mM glucose. The perfusate was kept at 37°C and aerated with a gas mixture of 95% O₂ + 5% CO₂. Myocardial contractile force (MCF) was isometrically...
measured by means of a force-displacement transducer (Nihon Kohden, TB-611T) connected to the apex of the heart through a thread. Coronary perfusion pressure (PP) was monitored by an electric manometer (Nihon Kohden, TP-101T) connected to a side-branch of the aortic cannule. Spontaneous heart rate (HR) was continuously counted with a cardiotachometer (Nihon Kohden, AT-600G) triggered by the signal of MCF. All parameters were recorded on a heat-writing recorder (Nihon Kohden, WT-645G). The basal tension was set at about 1.0 g and PP set at 50 mmHg by controlling the infusion flow rate. Experiments were started after these parameters reached a steady state.

In this experiment, drugs used were atenolol (ICI), propranolol hydrochloride (ICI), metoprolol tartrate (Ciba-Geigy), timolol maleate (Merck-Banyu) and nor-epinephrine hydrochloride (Sankyo). Drugs were dissolved and diluted with distilled water. The doses of these beta-adrenoceptor blocking agents were chosen by determining the doses at which they showed equipotent effects to that of propranolol in beta-adrenoceptor blockade (14–16). Doses of drugs administered were represented in terms of 'g' of the free form in atenolol of the salt in other drugs. Drug solutions were injected into a rubber tube in the perfusion apparatus in a volume of 0.1 ml or infused at a rate of 0.1 ml/min by means of an infusion pump (Harvard, 975 or 2620D). The vehicle had no effect on each parameter measured except for artifacts of injection.

First, cumulative administration of NE was repeated before and 10 min after bolus injection or during infusion of each beta-adrenoceptor blocking agent. Repeated administration of NE alone produced almost the same responses in each parameter as in the first administration. Second, during infusion of NE, bolus injection of two selected beta-adrenoceptor blocking agents, atenolol and propranolol, was performed.

The data were expressed as means±S.E. of percent changes from the predrug values of each parameter and analyzed by Student’s t-test.

Results

Cumulative injection of 10^{-8}–10^{-6} g NE produced a fall in PP and increases in MCF and HR in a dose-dependent manner as shown in Fig. 1. Bolus injection of 3×10^{-6} g atenolol 10 min before the second injection of NE did not obviously affect NE-induced changes in each parameter (Fig. 1). Bolus injection of 10^{-7}–3×10^{-6} g atenolol (n=9, Fig. 2A) and 10^{-7}–3×10^{-6} g metoprolol (n=6, Fig. 2B) did not significantly affect percent

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Fig. 1. A typical recording of the effect of bolus administration of atenolol on norepinephrine (NE)-induced responses in isolated perfused rat hearts.
changes in MCF and HR induced by cumulative administration of $10^{-8} - 10^{-6}$ g NE, although only PP responses to NE after a high dose of $3 \times 10^{-6}$ g atenolol or $3 \times 10^{-6}$ g metoprolol were significantly inhibited.

As seen in Fig. 3A, bolus injection of $3 \times 10^{-8} - 3 \times 10^{-7}$ g timolol (n=6) did not significantly inhibit dose-related percent increases
in MCF and HR by $10^{-8}-10^{-6}$ g NE, except for significant inhibition of PP by a high dose of $3 \times 10^{-7}$ g timolol. On the other hand, bolus injection of $10^{-7}-3 \times 10^{-6}$ g propranolol ($n=9$) significantly inhibited NE-induced responses of all parameters dose-dependently (Fig. 3B).

When infusion of atenolol at $10^{-6}$ g/min ($n=7$, Fig. 4A) or metoprolol at $10^{-6}$ g/min ($n=7$, Fig. 4B) was started at 10 min before the second application of NE, dose-dependent responses of PP, MCF and HR to $3 \times 10^{-9}-10^{-6}$ g NE were significantly inhibited. The lower dose of atenolol, $10^{-7}$ g/min ($n=7$), did not show a significant inhibitory effect on

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**Fig. 3.** Effects of bolus injection of timolol (A; $n=6$) or propranolol (B; $n=9$) on NE-induced changes in PP, MCF and HR in isolated perfused rat hearts. Open circles: control, closed circles: after $3 \times 10^{-8}$ g timolol (A) or $10^{-7}$ g propranolol (B), open squares: after $10^{-7}$ g timolol (A) or $3 \times 10^{-7}$ g propranolol (B), closed squares: after $3 \times 10^{-1}$ g timolol (A) or $10^{-6}$ g propranolol (B), open triangles: after $3 \times 10^{-6}$ g propranolol (B). Each point indicates means±S.E. of percent change. 100% is 48.4±0.5 (A) and 50.4±0.4 (B) mmHg for PP, 2.87±0.09 (A) and 2.70±0.37 (B) g for MCF and 253±7 (A) and 199±12 (B) beats/min for HR. *$P<0.05$ and **$P<0.01$ vs. the corresponding values of the control.
NE-induced responses. Infusion of timolol at $10^{-7}$ g/min (n=7) or propranolol at $10^{-7}$-$10^{-6}$ g/min (n=7) also significantly inhibited percent changes in all parameters induced by cumulative administration of $3\times10^{-9}$-$10^{-6}$ g NE (Fig. 5A and 5B).

Infusion of NE at $3\times10^{-8}$ g/min produced sustained increase in MCF, which reached 125–130% of the predrug value and persisted for more than 30 min. Individual bolus injection of atenolol at the dose of $10^{-7}$, $3\times10^{-7}$, $10^{-6}$ or $3\times10^{-6}$ g (n=7) transiently inhibited the increase in MCF, and its inhibition recovered toward the predrug level within 5 min (Fig. 6A). On the other hand, the inhibitory effect of bolus injection of propranolol at the dose of $10^{-7}$ or $3\times10^{-7}$ g (n=7) on NE-increased MCF continued for more than 10 min (Fig. 6B).

**Fig. 4.** Effects of infusion of atenolol (A; n=7) or metoprolol (B; n=7) on NE-induced changes in PP, MCF and HR in isolated perfused rat hearts. Open circles: control, closed circles: during $10^{-7}$ g/min atenolol (A) or $10^{-6}$ g/min metoprolol (B). open squares: during $10^{-6}$ g/min atenolol. Each point indicates means±S.E. of percent change. 100% is 49.5±0.4 (A) and 49.9±0.6 (B) mmHg for PP, 3.70±0.24 (A) and 2.90±0.32 (B) g for MCF and 245±8 (A) and 226±9 (B) beats/min for HR. *P<0.05 and **P<0.01 vs. the corresponding values of the control.
Fig. 5. Effects of infusion of timolol (A; n=7) or propranolol (B; n=7) on NE-induced changes in PP, MCF and HR in isolated perfused rat hearts. Open circles: control, closed circles: during 10^{-7} g/min timolol (A) or 10^{-6} g/min propranolol (B), open squares: during 10^{-6} g/min propranolol. Each point indicates means±S.E. of percent change. 100% is 48.5±0.4 (A) and 50.1±0.3 (B) mmHg for PP, 2.75±0.18 (A) and 3.80±0.33 (B) g for MCF and 233±8 (A) and 232±9 (B) beats/min for HR. *P<0.05 and **P<0.01 vs. the corresponding values of the control.

Discussion

In this study, NE was used as a cardioacceleratory agonist, since NE has been established to be a neurotransmitter released from sympathetic nerve endings (17). The present results showed that NE produced a fall in PP and increases in MCF and HR, probably through an activation of beta_1-adrenoceptors (18, 19). By bolus injection, atenolol, a beta_1-adrenoceptor blocking agent, did not significantly inhibit such NE-effects. Bolus injection of metoprolol, a similar beta_1-adrenoceptor blocking agent, also did not show significant inhibition on NE responses. Since it has been reported that there exist beta_1 and beta_2 adrenoceptors in rat heart (20, 21) and it cannot be completely ruled out that NE has a beta_2-adrenoceptor activating effect in addition to beta_1-adrenoceptor activation, effects of timolol, a nonselective adrenoceptor blocking agent, were examined in this study.
Bolus injection of timolol, like atenolol or metoprolol, did not significantly inhibit cardiac responses to NE.

Pharmacological properties of beta-adrenoceptor blocking agents are characterized by their intrinsic sympathomimetic activity, membrane stabilizing activity, protein binding and/or lipophilicity in addition to their selectivity to beta-adrenoceptor subtypes (14–16, 22, 23). Atenolol, metoprolol and timolol have been considered to be 'low lipophilic' (22, 23). Thus, it was predicted that bolus injection of these three drugs with low lipophilicity might be ineffective in inhibiting NE-responses under the present experimental conditions. To confirm this hypothesis, effects of bolus injection of highly lipophilic propranolol (22, 23) or effects of infusion of three drugs with low lipophilicity, atenolol, metoprolol and timolol, were examined in this study. Both bolus injection of propranolol and infusion of atenolol, metoprolol and timolol significantly inhibited NE-induced responses. Infusion of propranolol showed complete inhibition on NE responses.

Figure 7 shows the relation between the lipophilicity and the potency of inhibition by bolus administration or infusion of each beta-adrenoceptor blocking agent on MCF increased by $10^{-7}$ g NE. Propranolol showed a similar degree of inhibition by either bolus injection or infusion. Bolus injection of drugs with low lipophilicity such as atenolol, metoprolol and timolol showed no or very weak inhibition on $10^{-7}$ g NE-increased MCF, while potencies of inhibition by infusion of these drugs were equal to that of propranolol, supporting the hypothesis that bolus injections of atenolol, metoprolol and timolol are ineffective for inhibiting NE-response because of
Fig. 7. The relation between the lipophilicity and the inhibition rate by bolus administration or infusion of each beta-adrenoceptor blocking agent on MCF increased by bolus application of $10^{-7}$ g NE. Open symbols: after bolus injection of each beta-adrenoceptor blocking agent, closed symbols: during infusion of each beta-adrenoceptor blocking agent. Circles: atenolol, squares: metoprolol, stars: timolol, triangles: propranolol.

their low lipophilicity.

The reason why bolus injection of high doses of beta-adrenoceptor blocking agents with low lipophilicity significantly inhibited a fall in PP induced by cumulative administration of NE was not clarified in the present study. One possibility is that coronary arteries responded to beta-adrenoceptor blocking agents with low lipophilicity more strongly than myocardium, which was not clarified in this study.

In conclusion, the present results indicated that propranolol with high lipophilicity inhibited NE-induced responses in isolated hearts by both bolus injection or infusion, while drugs with low lipophilicity such as atenolol, metoprolol and timolol were ineffective in inhibiting NE-responses by bolus injection but effective by infusion, suggesting that beta-adrenoceptor blocking agents have different time course of action on NE-responses of isolated and perfused rat hearts through their different properties, especially the difference of lipophilicity.

Acknowledgments: The authors are grateful to ICI Pharma Co. for the kind gift of atenolol and propranolol, to Ciba-Geigy Co. for metoprolol and to Merck-Banyu Co. for timolol; and we would like to acknowledge Miss Yuko Kinjo for preparing this manuscript.

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