CARDIAC EFFECTS OF KETANSERIN, A SEROTONIN ANTAGONIST — ELECTROPHYSIOLOGICAL EXAMINATIONS AS A PART OF TOXICITY STUDIES —

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Abstract.....Cardiac effects of ketanserin were examined mainly electrophysiologically with using rat and guinea pig heart muscle preparations. 10^{-6} M ketanserin slightly antagonized the positive chronotropic but not inotropic action of serotonin in spontaneously beating guinea pig atria. Ketanserin, only at the concentration as high as 10^{-4} M, produced slight rightward shift of the positive chronotropic but not inotropic dose-response curves for norepinephrine in guinea pig atria. In both rat and guinea pig atria, ketanserin *per se* produced negative chronotropic effect and slight prolongation of action potential duration (APD) at high concentrations, 0.1 or 0.3mg / ml. In guinea pig ventricular preparation, 1mg / ml of ketanserin did not affect the rate of rise of the action potential ($+\dot{V}_{\max}$), action potential amplitude and APD. In rat ventricular free wall preparations, 1mg / ml of ketanserin produced slight increase in APD without affecting the other action potential parameters. In rat ventricular papillary muscle and septum preparations, 0.3 mg / ml of ketanserin tended to produce a decrease in $+\dot{V}_{\max}$ and an increase in APD. However, since these changes were produced only at extremely high concentrations and slight in degree, it was concluded that ketanserin does not produce electrophysiological side effects of clinical relevance.

Key words : Ketanserin, serotonin antagonist, cardiac action potential, cardiac toxicity,

INTRODUCTION

Ketanserin is a serotonin antagonist developed recently. It is considered to be a selective antagonist of 5-HT₂ receptors (Cohen, et al., 1981 ; Leysen, et al., 1981 ; Van Nueten, et al., 1983). Ketanserin shows antihypertensive effects and is believed to exert its hypotensive effects mainly by blocking the vasoconstrictor effect of

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serotonin on 5-HT₂ receptors (DeCrée, et al., 1981 ; Schalekamp, et al., 1986), although it has a weak alpha-adrenergic blocking activity, which may contribute to its hypotensive action (Marwood, et al., 1984). Thus, it is expected to be useful for the treatment of systemic hypertension, and clinical studies have already been started. In the clinical studies, there have been a few reported cases associated with electrocardiographic QT-prolongation (Bazan, et al., 1985), which might be related to ketanserin administration. Therefore, in the present study, as a part of toxicity studies on ketanserin, its cardiac effects were examined with using isolated guinea pig and rat heart muscle preparations ; experiments were performed mainly by measuring cardiac action potentials with intracellular microelectrodes.

MATERIALS AND METHODS

Female guinea pigs (250-300g) and rats (150-200g) were sacrificed and the hearts were removed. Spontaneously beating atria, right ventricular free wall and papillary muscle preparations were obtained from guinea pig hearts. From rat hearts obtained were the preparations of spontaneously beating atria, right ventricular free wall, ventricular septum, and left ventricular papillary muscle. For mechanical experiments, spontaneously beating atria were suspended vertically in the organ bath containing 10ml physiological salt solution of the following composition (mM) : NaCl 135, KCl 3, CaCl₂ 2, MgCl₂ 1, NaHCO₃ 15, and glucose 5.5. The physiological salt solution was maintained at 37°C and bubbled with 95%O₂-5%CO₂. Contractions were measured with a force displacement transducer (Tokyo Baldwin) and recorded on an ink-writing oscillograph (Nihon Kohden, Model WI-621G). Norepinephrine (NE) or serotonin (5-HT) were added cumulatively to the bath in the presence or absence of ketanserin (10⁻⁴M for NE and 10⁻⁶M for 5-HT).

For electrical experiments, each preparation was pinned down horizontally on a cork block at the bottom of organ bath containing 3ml physiological salt solution of the above composition at 37°C and bubbled with 95%O₂-5%CO₂. Preparations were driven by an electric current pulses (1 Hz, 5msec, supramaximal) through bipolar platinum electrodes. Conventional microelectrode technique was utilized and the cardiac action potentials were obtained by microelectrode preamplifier with capacity neutralization (Nihon Kohden, MEZ-8101). After passing through a transient memory, they were recorded on an ink-writing oscillograph (Nihon Kohden, RJG-3004). Ketanserin was dissolved in distilled water at high concentrations, and small aliquot was added to the organ bath to give desired final concentrations.

Ketanserin tartrate was generously supplied by Kyowa Hakko Kogyo Co., Ltd.

RESULTS

1) Effects of Ketanserin on spontaneously beating atria

To examine the antagonism by ketanserin to 5-HT and NE, contractions of spontaneously beating atria were recorded, and positive chronotropic and inotropic responses were examined. 10^{-6} M ketanserin produced a slight rightward shift of the dose-response curve for positive chronotropic effect of 5-HT in rat atria; however, there was no change in the positive inotropic dose-response curves for 5-HT (Fig. 1a). Dose-response curves for NE were obtained in guinea pig atria. Ketanserin at a concentration as high as 10^{-4} M produced a slight rightward shift of the positive chronotropic dose-response curve for NE without affecting the positive inotropic dose-response curve (Fig. 1b).

Fig. 1 Effects of ketanserin on positive chronotropic and inotropic responses to serotonin (a) and norepinephrine (b). Beating rate and contractile force were measured with spontaneously beating atria from rats (a) and guinea pigs (b), and dose-response curves for serotonin and norepinephrine were obtained before (solid line) and after (broken line) the addition of ketanserin.

Spontaneously beating atria were also subjected for the measurements of intracellular action potentials. Both in rat and guinea pig atria, high concentration of ketanserin produced a decrease in spontaneous beating rate (Fig. 2); this negative chronotropic action of ketanserin appeared to be dose-dependent, representative data being shown in guinea pig atria (Fig. 2b). High concentration of ketanserin also produced dose-dependent prolongation of action potential duration (APD) in spontaneously beating atria both from rats and guinea pigs; APD30, APD50 and APD90 (time required to achieve 30%, 50% and 90% repolarization, respectively) were all prolonged (Fig. 2, dotted line). For comparison, action potentials were also recorded in rat and guinea pig left atria driven electrically at a constant rate (1 Hz). Also in the electrically driven atria, ketanserin tended to prolong APD (Fig. 2, solid line); however, the prolongation was only slight compared to that in spontaneously beating atria both in rats and guinea pigs.

2) Effects of ketanserin on ventricular action potentials

Action potentials recorded from guinea pig right ventricular free wall and

Fig. 2 Effects of ketanserin on the atria from rats (a) and guinea pigs (b). Beating rate and action potential duration were measured with spontaneously beating (broken line) and electrically driven (solid line) atria. Ketanserin was added at the arrow (doses are indicated in the figure). Note that ketanserin produced a decrease in beating rate both in rat and guinea pig atria dose-dependently and that it produced a prolongation of action potential duration which was more prominent in spontaneously beating atria both in the cases of rat and guinea pig. Ordinate: beating rate, APD30 (30%-duration), APD50 (50%-duration) and APD90 (90%-duration) expressed as % of control value before the addition of ketanserin. Abscissa: time (min) after the addition of ketanserin (1st dose).

papillary muscle were not significantly affected by 10^{-3} g / ml of ketanserin except for a slight shortening of the action potential duration (Fig. 3) ; there was no change in maximum rate of rise ($+\dot{V}_{\max}$) and amplitude of action potential. Lower concentrations did not affect the action potential of guinea pig ventricular muscle at all.

Fig. 3 Effects of ketanserin (1 mg / ml) on various parameters of action potentials recorded from guinea pig right ventricular free wall preparations. Maximum rate of rise ($+\dot{V}_{\max}$), amplitude of action potential, APD30 (30%-duration), APD50 (50%-duration) and APD90 (90%-duration) were measured. Changes in these parameters were expressed as % of control value (ordinate) before the addition of ketanserin as a function of time (min, abscissa).

Ketanserin even at high concentrations of 10^{-4} - 10^{-3} g / ml did not affect the action potential configuration of rat right ventricular free wall preparations ; $+\dot{V}_{\max}$ and the amplitude of action potential were not affected significantly (Fig. 4a). However, 10^{-3} g / ml of ketanserin tended to increase the action potential duration slightly (Fig. 4b). Extremely high concentration, 3×10^{-3} g / ml, was also tested, and it was found that this high concentration of ketanserin produced considerable decrease in $+\dot{V}_{\max}$ (to $30.4 \pm 11.2\%$) with slight decrease in the amplitude of action potential (to $73.3 \pm 9.1\%$), which was also accompanied by the increase in action potential duration, especially in APD30 (to $169.0 \pm 44.2\%$).

When 3×10^{-4} g / ml of ketanserin was applied to papillary muscle preparations obtained from rat left ventricle, slight decrease in $+\dot{V}_{\max}$ was observed without changes in the amplitude of action potential. This concentration of ketanserin also produced a slight prolongation of the action potential duration (especially in APD30 and APD50) transiently for several min after the addition of the drug ; however, APD90 did not change significantly (Fig. 5).

Effects of ketanserin was also examined on the action potentials recorded from rat ventricular septum. 3×10^{-4} g / ml of ketanserin produced a decrease in $+\dot{V}_{\max}$ of the action potential, which was slightly more marked than in the case of papillary muscle ; however, the action potential amplitude was little affected (Fig. 6).

Fig. 4 Electrophysiological effects of ketanserin on rat ventricular free wall preparations. Parameters measured were $+V_{\max}$ (a), action potential amplitude (a), APD30 (30%-duration, b), APD50 (50%-duration, b) and APD90 (90%-duration, b). Changes in these parameters (expressed as % of control value before the addition of ketanserin) were plotted (ordinate) as a function of time (min, abscissa). 3 doses of ketanserin (0.1, 0.3 and 1mg / ml) were tested, which are indicated on the top of each panel.

Cardiac effects of ketanserin

Fig. 5 Electrophysiological effects of ketanserin (0.3 mg / ml) on rat right ventricular papillary muscle preparations. Action potential parameters measured were $+V_{\max}$, action potential amplitude, APD30 (30%-duration), APD50 (50%-duration), and APD90 (90%-duration). Changes in these parameters (% of control value before the addition of ketanserin) were plotted (ordinate) as a function of time (min, abscissa).

Fig. 6 Electrophysiological effects of ketanserin (0.3 mg / ml) on rat ventricular septum. Action potential parameters measured were $+V_{\max}$, action potential amplitude, APD30 (30%-duration), APD50 (50%-duration), and APD90 (90%-duration). Changes in these parameters (% of control value before the addition of ketanserin) were plotted (ordinate) as a function of time (min, abscissa).

This concentration of ketanserin simultaneously increased the action potential duration ; in contrast to rat papillary muscle, all of APD30, APD50 and APD90 were prolonged, and it appeared that the prolongation of APD lasted for slightly longer than in the case of papillary muscle.

DISUSSION

Serotonergic receptors have been divided into 2 subclasses, 5-HT₁ and 5-HT₂. Relatively little is known about the distribution of serotonergic receptors in the heart. In the present study, ketanserin, a 5-HT₂-antagonist, produced rightward shift of the dose-response curve for positive chronotropic response to 5-HT in spontaneously beating rat atria. This finding might suggest that the chronotropic effect of 5-HT is mediated through 5-HT₂ receptors present in rat atria. However, the concentration of ketanserin to produce such antagonism was considerably high, and our preliminary experiments indicated that cyproheptadine, at a concentration as low as 10^{-9} M, produced much greater rightward shift of the chronotropic dose-response curve for 5-HT in rat atria. Therefore, 5-HT₂ receptor is unlikely to play predominant role in the positive chronotropic response to 5-HT. Moreover, ketanserin did not antagonize the positive inotropic response to 5-HT even at high concentrations. From these results, it may be considered that functional 5-HT₂ receptor is not present in rat atria.

Ketanserin antagonized the positive chronotropic response to NE in guinea pig atria, which may serve an evidence for the weak alpha-adrenergic blocking activity of ketanserin ; however, the concentration of ketanserin to produce such effect was again considerably high. With respect to the possible alpha-adrenergic blocking activity of ketanserin in the heart, it was reported that ketanserin-induced cardiac actions are not mediated by adrenergic alpha mechanisms (Furukawa, et al., 1986). Although further experiments are needed to clarify this point, it is not the aim of the present toxicological study. Therefore, we conducted no further experiments.

Ketanserin produced the decrease in spontaneous beating rate of the atria both of rats and guinea pigs. It also produced the prolongation of APD, and the prolongation of APD was more marked in spontaneously beating atria than in those driven electrically at a constant rate both in the cases of rat and guinea pig atria. Although the precise reason for this is unknown, the decreased rate might additively prolong the APD. Anyhow, since the concentrations of ketanserin to produce such changes were as high as 10^{-4} g / ml, the changes do not seem to be of clinical importance.

Guinea pig ventricular preparation is widely utilized for the electrophysiological studies with microelectrodes. Ketanserin did not produce substantial changes in the action potential configuration recorded from right ventricular free wall preparation and papillary muscle even at the concentration as high as 10^{-3} g / ml. Only an effect produced by high concentrations of ketanserin was slight reduction in APD. Thus,

it was concluded that, as far as studied electrophysiologically, ketanserin has no direct cardiac action on guinea pig ventricular muscle.

It was reported that the sensitivity of guinea pig myocardium to serotonin is relatively low. For example, we have reported that, although 10^{-5} M histamine produced almost maximum positive inotropic effects on guinea pig papillary muscle (about 60% increase from control level), serotonin produced only small increase in contractile force (about 10%) even at the concentration as high as 10^{-4} M (Tatsuno-Atoda, et al., 1981). Therefore, it is possible that the lack of ketanserin effect in guinea pig myocardium might be due to the low sensitivity of this muscle to serotonin. Therefore, effects of ketanserin was decided to be examined in rat myocardium.

The action potential configuration of rat myocardium is known to be quite different from that of other mammalian myocardium, like human and guinea pig, etc. The largest difference is in plateau component, i. e., the plateau component of rat ventricular action potential is extremely short; if compared the action potential duration, APD50 and APD30 of rat ventricular action potential are roughly 1/16 and 1/20 of that of guinea pig ventricular action potential, respectively. In addition, it is also well known that the sensitivity of rat myocardium to cardiac glycosides is extremely low. From these and other reasons, rat myocardium is generally considered not to be suitable for the electrophysiological and other experiments. However, the sensitivity to serotonin of rat myocardium is higher than that of guinea pig myocardium (unpublished observation). Therefore, in the present study, we decided to use rat myocardium for studies on the electrophysiological effects of ketanserin.

Thus, the effects of ketanserin were examined with using right ventricular free wall, ventricular septum, and left ventricular papillary muscle preparations obtained from rat heart. In right ventricular free wall preparation, ketanserin did not affect the amplitude and $+\dot{V}_{\max}$ of the action potential at concentrations from 10^{-4} to 10^{-3} g / ml, although APD tended to be increased by the highest concentration, 10^{-3} g / ml. In ventricular septum and papillary muscle, 3×10^{-4} g / ml of ketanserin produced a decrease in $+\dot{V}_{\max}$ and the prolongation of action potential duration without affecting action potential amplitude; and the changes were somewhat greater in ventricular septum than in papillary muscle. The difference between these preparations is unknown, but it may be possible, though highly speculative, that ventricular septum preparation may have abundant Purkinje fiber compared to other preparations. Therefore, it is possible that ketanserin, at extremely high concentrations, may produce the decrease in conduction velocity of specialized conduction system by decreasing the rate of rise of action potential. However, the concentrations of ketanserin to produce such effects were extremely high and is definitely unphysiological. Therefore, it is quite reasonable to conclude that ketanserin do not produce any electrophysiological side effects on cardiac muscle at least at the concentrations of clinical relevance.

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