Cardiovascular Effects of Orally Administered HNS-32, an Originally Synthesized Azulene-1-carboxamidine Derivative, Assessed in the In Vivo Rat Model

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ABSTRACT—HNS-32, an azulene-1-carboxamidine derivative, is an originally synthesized antiarrhythmic compound. Its cardiovascular effects after oral administration (1 – 10 mg/kg) were assessed using the pentobarbital-anesthetized in vivo rat model in comparison with those of verapamil (3 mg/kg, p.o.). Verapamil decreased the heart rate and mean blood pressure and prolonged the PR interval without changing the QRS width (n = 6). Similar results were observed for HNS-32 except that the QRS width was prolonged by the highest dose and the effects occurred slowly and lasted longer. These results suggest that HNS-32 is an orally active slowly-acting calcium plus sodium channel blocker.

Keywords: HNS-32, Azulene, Cardiovascular effect

An azulene-1-carboxamidine derivative (HNS-32), N1,N1'-dimethyl-N2-(2-pyridylmethyl)-5-isopropyl-3,8-dimethylazulene-1-carboxamidine, is an originally synthesized antiarrhythmic compound (1). It has been shown to inhibit sodium and calcium channels of guinea pig myocardial cells (2, 3), to suppress the sinus nodal automaticity and ventricular contractile force of the isolated guinea pig and canine hearts (1, 3), and to inhibit the atrioventricular nodal and intraventricular conduction of isolated canine hearts (1). This drug has been shown to inhibit both L-type calcium channel-dependent and -independent vascular contraction of pig coronary artery and rabbit aorta (4, 5), to increase the coronary blood flow of the isolated canine heart (1), and to exert antiarrhythmic effects against canine and rat ventricular arrhythmia models (1, 2). Since information regarding the in vivo effects of orally administered HNS-32 on the normal cardiovascular system is still lacking, in the present study, we assessed them in comparison with those of verapamil using the in vivo rat model (2, 6).

Animals were obtained through the Animal Laboratory for Research of Yamanashi Medical University. All experiments were performed in accordance with Guidelines for Animal Experiments of Yamanashi Medical University.

Male Sprague-Dawley rats weighing 200 – 300 g were anesthetized with 60 mg/kg of intra-peritoneal injection of pentobarbital sodium (2, 6). A heparinized catheter was placed in the aorta via the left carotid artery for continuous monitoring of the systemic blood pressure. The surface lead II ECG was obtained from the limb electrodes. The ECG together with the systemic blood pressure was continuously monitored using a polygraph system (RM-6200; Nihon Kohden, Tokyo) and recorded on a thermal recorder (WS-682G, Nihon Kohden) at a paper speed of 200 mm/s. After the assessment of the basal control state, HNS-32 (1, 3 and 10 mg/kg), verapamil (3 mg/kg) or the solvent saline was administered in a volume of 1 ml/kg directly into the stomach using a gastric tube. Effects of the drugs on the PR interval and QRS width of ECG, heart rate, and mean blood pressure were assessed every 10 min over 190 min.

HNS-32 was synthesized in the Department of Chemistry, Yamanashi Medical University. The following drugs were purchased: pentobarbital sodium (Tokyo-Kasei, Tokyo), heparin calcium (Mitsui, Tokyo) and verapamil hydrochloride (Sigma, St. Louis, MO, USA). 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 analysis of variance (ANOVA) followed by Contrast for statistical analysis between basal values and others, whereas those between the groups were assessed using one-way Factorial ANOVA. A P-value less
than 0.05 was considered significant.

Each control value was not significantly different among the five groups; namely, 1, 3 and 10 mg/kg of HNS-32 administered groups, 3 mg/kg of verapamil administered group and saline administered groups. While the control value of QRS width of the verapamil group tended to be shorter than that of control group, the difference did not achieve statistical significance (Fig. 1). After the administration of saline, no significant change was detected in any parameter during the observation period (n=6), as shown in Figs. 1, 2 and 3 (closed symbols), indicating the electrophysiological and hemodynamic stability of the currently used rat in vivo model.

The time courses of the effects of HNS-32 and verapamil on the ECG parameters are summarized in Fig. 1 (n=6 for each group). No significant changes were observed in the PR interval and QRS width after the administration of the low dose of HNS-32. The middle dose of HNS-32 prolonged the PR interval without changing the QRS width. The high dose of HNS-32 prolonged the PR interval and QRS width. On the other hand, verapamil transiently prolonged the PR interval without changing the QRS width.

The time courses of the effects of HNS-32 and verapamil on the heart rate and mean blood pressure are summarized in Figs. 2 and 3. The low dose of HNS-32 decreased the heart rate without changing the mean blood pressure. The middle and high doses of HNS-32 decreased the heart rate and mean blood pressure. Verapamil decreased the heart rate and mean blood pressure.

Given the limited information regarding the in vivo cardiovascular effects of orally administered HNS-32, we assessed them in comparison with those of verapamil using the in vivo rat model (2, 6). As shown in this study, orally administered verapamil decreased the heart rate and mean blood pressure and prolonged the PR interval, while no significant change was detected in the QRS width. These results are essentially in accordance with previous reports of verapamil from in vitro and in vivo experiments (7, 8). Also, orally administered HNS-32 decreased the heart rate and mean blood pressure and increased the PR interval, which is qualitatively and, if we compare the peak effects, is quantitatively similar to verapamil. This may indicate that orally administered HNS-32 blocks the calcium channels in vivo. In addition, HNS-32 slightly but significantly prolonged the QRS width with its highest dose, suggesting that this compound may also have a sodium channel blocking property in vivo, since intraventricular conduction solely depends on the sodium current (9). It should be also noted that the peak cardiovascular effects of HNS-32 occurred slowly and the effects lasted longer compared to verapamil, suggesting that some metabolites of HNS-32 may exert such effects, or that the distribution of HNS-32...
to the heart can be relatively slow (2).

In summary, the present results support the previous in vitro report that HNS-32 blocks the sodium channel in addition to the calcium channel (3), and they suggest that

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**Fig. 2.** The time course of the effects of HNS-32 and verapamil on the heart rate (HR) (open circles, n = 6 for each group). Closed circles in each panel indicate the results of saline (n = 6). The data are presented as the mean ± S.E.M. *P<0.05 vs pre-drug control value (C).

**Fig. 3.** The time course of the effects of HNS-32 and verapamil on the mean blood pressure (MBP) (open circles, n = 6 for each group). Closed circles in each panel indicate the results of saline (n = 6). The data are presented as the mean ± S.E.M. *P<0.05 vs pre-drug control value (C).
HNS-32 may become an orally active slowly-acting calcium plus sodium channel blocker for the treatment of various arrhythmias.

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REFERENCES


