Inotropic Effects of (±)-Higenamine and Its Chemically Related Components, (+)-R-Coclaurine and (+)-S-Reticuline, Contained in the Traditional Sino-Japanese Medicines "Bushi" and "Shin-i" in Isolated guinea Pig Papillary muscle

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Abstract—(±)-Higenamine (Hig, demethylcoclaurine) is a cardiotonic principle from aconite root. (+)-R-Coclaurine (Coc) and (+)-S-reticuline (Ret) are compounds contained in the dried buds of Magnolia salicifolia MAXIM. All of these alkaloids possess a common chemical structure: tetrahydroisoquinoline. Coc and Ret showed negative inotropic effects in contrast to the positive inotropic effects of Hig in papillary muscles of guinea pigs. Coc and Ret shifted to the right the concentration-contraction curves of Hig. Hig shifted in parallel to the left the Ca2+ curve, and it tended to shift to the left the isoproterenol (Isp)-induced response curve. In contrast, Coc and Ret inhibited the Ca2+ curve and the low concentration range of the Isp-induced curve, and it potentiated the high concentration ranges of Ca2+ and Isp. Coc and Ret showed actions that were reversed in direction to those of Hig, as clearly demonstrated in the Ca2+ curve.

(±)-Higenamine (Hig, demethylcoclaurine) is a cardiotonic principle contained in the traditional Sino-Japanese medicine "Bushi" (Aconite root) (1). The cardiovascular pharmacological properties and the clinical applications of Hig have been reviewed by Chou (2). Hig was effective on heart failure (3), was reported to be a β-adrenergic agonist (4), and to enhance Ca2+ influx (5, 6).

(+)-R-Coclaurine (Coc) and (+)-S-reticuline (Ret) are compounds contained in the traditional Sino-Japanese medicine "Shin-i" (the dried buds of Magnolia salicifolia MAXIM). Coc, Ret and Hig all possess the common chemical skeleton: 1-benzyl-1,2,3,4-tetrahydroisoquinoline (Fig. 1). The cardiovascular effects of Coc and Ret have not yet been reported. Therefore, Hig, and Coc and Ret were compared with the

Fig. 1. The Chemical structures of (±)-higenamine, (+)-R-coclaurine and (+)-S-reticuline.
effects on the isoproterenol (Isp) - and CaCl₂-induced positive inotropic actions. Furthermore, the effects of Coc and Ret on Hig-induced action were also investigated.

Guinea pigs (male), weighing 250-500 g, were stunned and the beating hearts were quickly removed from the thoraxes to a beaker containing Krebs-Henseleit solution of the following composition: 118.4 mM NaCl, 4.69 mM KCl, 2.0 mM CaCl₂, 1.16 mM MgCl₂, 1.18 mM KH₂PO₄, 24.9 mM NaHCO₃ and 5.0 mM glucose (KH₂PO₄ was omitted when determining the CaCl₂ concentration-response curve). The papillary muscles with width between 0.5 and 1.0 mm of either the right or left ventricles were rapidly excised from the isolated hearts and mounted in an organ bath containing 2.5 ml of the above nutrient solution at 30±1 °C, gassed with 95% O₂ and CO₂, resulting in a pH of 7.7. The papillary muscles with 0.5 g initial tension were equilibrated for about 1 hr under the stimulation of a square wave pulse (voltage: 10 V, duration: 0.5 msec, frequency: 0.35 Hz, via bipolar platinum electrodes). The contraction force was measured isometrically by means of a force transducer (Shinkoh Tsushin, U-Gage, Type UL 2-240, Minebea) and recorded on a pen-writing oscillograph (Nihon Kohden Kougyou). (+)-Isoproterenol HCl (Kaken), (±)-higenamine HBr, (+)-R-coclaurine HCl and (+)-S-reticuline oxalate were used. All drugs were dissolved in distilled water. Student’s t-test was used for statistical analysis, with P<0.05 taken to indicate a significant difference.

Hig showed positive inotropic actions in a concentration-dependent manner from 28 nM to 53.9 µM (Fig. 2b, upper). The maximum positive inotropic response induced by Hig was 117.5±6.6% (n=7) for the maximum response to Isp. The maximum response for CaCl₂ was 121.7±15.2% (n=7). Hig at 280 nM tended to potentiate the concentration-positive inotropic action curve induced by Isp in the low concentration range of Isp (data not shown). Hig (280 nM) shifted the concentration-response curve of CaCl₂ in parallel to the left (Fig. 2b lower, right).

Coc and Ret (0.25–0.50 mM), with 10 min treatment, induced the negative inotropic actions by 8.4±1.3% to 9.7±2.3% (n=6) and by 10.2±2.6% to 12.3±2.0% (n=6–8), respectively (the maximum contraction induced by Isp (258 nM) was taken as 100%). At 10 min after their weakly negative inotropic actions were saturated, Isp (Fig. 2a), Hig (Fig. 2b upper) and CaCl₂ (Fig. 2b lower) were cumulatively added. Coc and Ret inhibited the positive inotropic action of Isp at the low concentrations and potentiated that at high concentrations of Isp. Ret at 0.25 mM exerted a stronger inhibitory effect on the low concentrations of Isp than did Coc, while Coc at 0.50 mM produced greater potentiation for the high concentrations of Isp than did Ret.

Coc at 0.50 mM significantly shifted, apparently in parallel to the right, the concentration-contraction curve for Hig, which was confirmed by the double reciprocal plot (Fig. 2b upper, left). Ret at 0.125–0.50 mM inhibited it markedly and non-competitively. Coc and Ret at 0.50 mM produced biphasic effects composed of inhibition at low concentrations and of weak potentiation at high concentrations in the CaCl₂ curves.

The above results have demonstrated that Coc and Ret show a negative inotropic action in contrast to the positive inotropic action of Hig and also demonstrated their antagonistic actions to Hig. The chemical structure of Coc is only different from that of Hig in that it has a methoxy group at the 6-position of the tetrahydroisoquinoline skeleton, while Hig has a hydroxyl group at this position. The chemical structural difference caused the reversed direction of inotropic action.

The cardiotonic effect of Hig was ascribed to its β-adrenoceptor agonistic action because it was inhibited by propranolol (4). On the other hand, Hig (14.2 µM) was reported to enhance the inward slow Ca²⁺ current in porcine (5) and dog (6) ventricular myocardial fibers. In the present study, the positive inotropic action of Hig may be caused, at least in part, by the acceleration of slow Ca²⁺ influx. The Hig-induced positive inotropic response was inhibited by Coc in an apparently competitive manner.

In conclusion, the cardiac actions of Coc and Ret were reverse in direction to those caused by Hig. The hydroxy group of Hig at
Fig. 2. The effects of (+)-R-coclaurine (left, n=4-5) and (+)-S-reticuline (right, n=3-5) on cumulative concentration curves for a) isoproterenol (2.02-258 nM), b) (±)-higenamine (28 nM-79.5 μM, upper) and CaCl₂ (4-64 mM, lower), and the effect of (±)-higenamine (n=4, 0.28 μM) on the CaCl₂ curve in isolated papillary muscles of guinea pigs. Values are means±S.E.M. Significant differences from the control values before application of (±)-higenamine, (+)-R-coclaurine and (+)-S-reticuline: *P<0.05 and **P<0.01.
the 6-position of its tetrahydroisoquinoline skeleton is important for the production of a positive inotropic response. One of the action mechanisms for Hig is related to the enhancement of Ca\(^{2+}\) influx.

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References