STUDIES ON A NEW 1, 5-BENZOTHIAZEPINE DERIVATIVE (CRD-401) V. ANTIARRHYTHMIC ACTIONS

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Abstract—The effect of CRD-401 on several types of experimentally induced arrhythmias in guinea-pigs and dogs and the antagonistic effect against isoproterenol in the isolated atrium of guinea-pigs were studied and compared with quinidine and propranolol. In epinephrine-induced arrhythmias of guinea-pigs, propranolol had a notable but brief depressive effect, which was observed only 5 min after administration of the drug. Although the potency of CRD-401 was weaker than that of propranolol in these arrhythmias, the effect persisted for more than 35 min after administration. In ouabain-induced arrhythmias of guinea-pigs, CRD-401 and propranolol showed similar depressive effects. Quinidine had little effect on both epinephrine- and ouabain-induced arrhythmias. Both CRD-401 and propranolol failed to reverse the arrhythmias produced by two-stage coronary artery ligation. Propranolol showed a competitive antagonism against the positive chronotropic effect of isoproterenol, while CRD-401 revealed a non-competitive β-blocking action. Quinidine produced no blocking effect in the present experiments.

CRD-401 or d-3-acetoxy-cis-2, 3-dihydro-5-[2-(dimethylamino) ethyl]-2-(p-methoxyphenyl)-1, 5-benzothiazepin-4 (5H)-one hydrochloride (Fig. 1) is a new benzothiazepine derivative with notable coronary vasodilating activity in anaesthetized dogs and in the isolated guinea-pig heart (1, 2). CRD-401 also exhibits a weak depressive effect, in large doses, on the beating rate and contractile force of the dog heart (1). In the present study, the antiarrhythmic action of CRD-401 was investigated and compared with that of propranolol and quinidine (3–5).

![Fig. 1. Structural formula of CRD-401.](image)
METHODS

I. Arrhythmias in anaesthetized guinea-pigs

Male guinea-pigs (Hartley strain) weighing 300 to 380 g were anaesthetized with urethane (1.0 g/kg i.p.). Electrocardiograms (ECGs) were taken from Lead II or Lead aVF. Blood pressure from a canulated carotid artery was monitored with an electric transducer and recordings were made on Nihon Kohden Twin writing oscillograph. Drugs were injected into the catheterized femoral vein after the heart rate and blood pressure had reached a steady level 100 to 120 min after anaesthesia.

I-1. Epinephrine-induced arrhythmias: arrhythmias were elicited by rapid i.v. injections of epinephrine 50 /ug/kg. This drug was administered three times at 30-min intervals to each animal. The initial response was obtained as control and a test drug was injected 5 min before the second administration of epinephrine. ECG tracings were analyzed according to the method of Maling et al. (6), in which the total heart rate and the rate of ectopic beats were counted for each successive 30-sec interval.

I-2. Ouabain-induced arrhythmias: arrhythmias were elicited by the infusion of ouabain (concentration of 7.5 ug/ml) at the rate of 0.33 ug/min into the femoral vein. Effects of the drugs were tested by administration 5 min before the infusion of ouabain and during initiation of the arrhythmia. The effect of prior administrations was determined by comparing the dose of ouabain which produced ventricular extrasystole, ventricular flutter or fibrillation, and cardiac arrest with that in control animals. Cardiac arrest was defined as such when asystole persisted over 3 sec.

II. Arrhythmias in dogs

Male mongrel dogs weighing 14 to 18 kg were anaesthetized with pentobarbital-Na (30 mg/kg i.v., supplemented by additional doses when required). The chest was incised and the two-stage coronary artery occlusion was carried out according to the method of Harris (7). Blood pressure was measured from the femoral artery (during anaesthesia) and ECGs were taken from Lead II throughout the experiment. CRD-401 or propranolol was administered to the animals during development of "spontaneous" ventricular arrhythmias 6 to 24 hr after coronary ligation. These animals were sacrificed 2 weeks after ligation and the hearts removed to investigate the size and grade of the infarcts.

III. Antagonistic effect against isoproterenol

Spontaneously beating right atria were prepared after the method by Black et al. (8). The preparations were suspended in a bath of the modified Tyrode's solution of the following composition: NaCl, 147 mM; KCl, 2.68 mM; CaCl₂, 1.8 mM; MgCl₂, 0.49 mM; Na₂HPO₄, 1.5 mM; Na₃HPO₄, 4.5 mM; glucose, 0.1% (pH 7.2). The bath was aerated with oxygen and kept at 30±1°C. A mechanical record was taken through the strain gauge (Shinko Tsushin) on the writing oscillograph and the rhythmic rate was counted. Under these conditions the rate was approx. 120 to 160 beats/min.

Cumulative dose-response curves were obtained by adding isoproterenol (10⁻¹⁰ to 10⁻³ g/ml) alone or 15 min after adding an antagonist (10⁻⁵ g/ml).
IV. Drugs

The following drugs were used: CRD-401 (synthesized by Tanabe Seiyaku), propranolol (Sumitomo Chemical), quinidine (Tokyo Kasei), epinephrine (Sankyo), isoproterenol (Böhringer Sohn), ouabain (Takeda).

RESULTS

I. Antiarrhythmic effect in guinea-pig

I-1. Epinephrine-induced arrhythmias

Cardiac irregularities after a rapid injection of epinephrine (50 μg/kg) usually lasted for 2 to 3 min and consisted mainly of A-V nodal rhythm, ventricular extrasystole or tachycardia, preceded by bradycardia or A-V block. Epinephrine injections were repeated at 30-min intervals in order to examine the change in response by repetition (Fig. 2). Although the character of arrhythmias was similar after each injection of epinephrine, the number of ectopic beats decreased and the duration of irregular beats shortened gradually in comparison with those of the previous injection.

These cardiac irregularities were effectively antagonized by propranolol or CRD-401 respectively. Propranolol (1.0 mg/kg) showed a maximum inhibition of arrhythmias 5 min after administration. At this time few or no ectopic beats were observed. Thirty-five min after administering propranolol, ectopic beats produced by epinephrine increased, the number of which was not statistically different from that of control (Fig. 3).

Although the effect of CRD-401 (1.0 mg/kg) 5 min after administration was less pronounced than that of propranolol, this inhibitory action continued even 35 min after administration (Fig. 4).

Fig. 2. Effect of repeated administrations of epinephrine. Epinephrine (50 μg/kg, each arrow) was injected three times at 30-min intervals. Each column shows heart rate per min for 30 sec (sinus beats, white portions; ectopic beats, hatched portions). Standard errors of the means are shown by vertical lines (10 experiments).
FIG. 3. Antagonistic effect of propranolol on epinephrine-induced arrhythmias (7 experiments). Epinephrine injections were repeated at 30-min intervals and propranolol was administered 5 min before the 2nd injection of epinephrine. Other explanations are the same as in Fig. 2.

FIG. 4. Antagonistic effect of CRD-401 on epinephrine-induced arrhythmias (7 experiments). Explanations are the same as in Figs. 2 and 3.

Quinidine (1.0 mg/kg) showed no protective effect on epinephrine arrhythmias (Fig. 5).

I-2. **Ouabain-induced arrhythmias**

The various types of arrhythmias induced by ouabain infusion were effectively reversed to the sinus rhythm, after i.v. administration of CRD-401 in doses of 0.5 to 2.0 mg/kg. The reversal persisted for 22 to over 120 min (Table 1).
FIG. 5. Antagonistic effect of quinidine on epinephrine-induced arrhythmias (7 experiments).

TABLE 1. Effect of CRD-401, propranolol and quinidine on ouabain arrhythmias.

| Test drugs   | Dose (mg/kg) | Dose* (mg/kg) | Type of arrhythmias** | Effect*** | Duration of effect (min****) |
|--------------|--------------|---------------|-----------------------|-----------|-----------------------------|
| CRD-401      | 2            | 0.12          | FLUT & FIBRIL         | Reversion | 6 to 123                    |
|              | 2            | 0.24          | EXTRA & TACHY         |           | 1 to 85                     |
|              | 2            | 0.07          |                       |           | 1 to 22                     |
|              | 1            | 0.10          | FLUT & FIBRIL         |           | 3 to 24                     |
|              | 1            | 0.14          | EXTRA & TACHY         |           | 7 to 30                     |
|              | 0.5          | 0.05          |                       |           | 1 to >120                   |
| Propranolol  | 2            | 0.14          | FLUT & FIBRIL         | Reversion | 3 to 89                     |
|              | 2            | 0.09          |                       | Transient | –                           |
|              | 2            | 0.28          | EXTRA & TACHY         | Reversion | 4 to 65                     |
| Quinidine    | 2            | 0.26          |                       | Transient | –                           |
|              | 1            | 0.23          |                       | Reversion | 1 to 60                     |
|              | 1            | 0.08          |                       |           | 1 to >60                    |
|              | 0.5          | 0.14          |                       | No effect | –                           |
|              | 2            | 0.20          | EXTRA & TACHY         | Transient | –                           |
|              | 2            | 0.12          |                       |           | –                           |
|              | 2            | 0.14          |                       | No effect | –                           |

* Dose of ouabain infused until the test drugs were administered
** FLUT & FIBRIL, Ventricular flutter and fibrillation;
   EXTRA & TACHY, Ventricular extrasystole and tachycardia
*** Reversion, reversion to the sinus rhythm
   Transient, transient reversion to the sinus rhythm
**** Time after administration of test drugs
Propranolol was also effective in ouabain arrhythmias. Quinidine was not effective in reversing arrhythmias to the sinus rhythm at 1.0 to 2.0 mg/kg.

The administration of propranolol (2 mg/kg) 5 min before ouabain infusion increased the dose of ouabain required to develop the ventricular extrasystole, flutter or fibrillation of ventricle and asystole (Fig. 6).

CRD-401 (2 mg/kg) also prevented various cardiac arrhythmias induced by ouabain, but potency was less than that of propranolol.

II. Arrhythmias produced by coronary artery ligation in dogs

Propranolol or CRD-401 was administered in doses of 1.0 to 2.0 mg/kg i.v. to dogs 6 to 24 hr after coronary artery occlusion. Both agents failed to reverse the arrhythmias produced by occlusion to the sinus rhythm.

The size and the color of the gross infarcted areas were almost the same between drug-treated and non-treated control animals.

III. Antagonism against isoproterenol

Fig. 7 shows the effects of quinidine, propranolol and CRD-401 on positive chronotropic responses to isoproterenol in the isolated guinea-pig right atria pre-incubated with the antagonists. Propranolol in a concentration of $10^{-7}$ g/ml shifted the dose-response curve for isoproterenol in a parallel fashion to the right, this shift being characteristic of competitive antagonists.

On the other hand, CRD-401 showed a non-competitive antagonistic effect; that is, $10^{-7}$ g/ml of CRD-401 depressed the maximum response of the atrium to isoproterenol...
Quinidine of $10^{-7}$ g/ml did not antagonize the effect of isoproterenol.

DISCUSSION

The character of arrhythmias produced by epinephrine (50 μg/kg) was similar to that reported by Somani et al. (9). These authors showed that repeated injections of epinephrine into dogs at 30-min intervals for over 2 and a half hr produced arrhythmias of a similar character and duration. In the present study with guinea-pigs, however, the ectopic response decreased gradually with the repeated administration of epinephrine. This difference may be due to animal species used.

In epinephrine-induced arrhythmias, the maximal antagonizing effect of CRD-401 was weaker than that of propranolol, but it was noted that the duration of the effect of the former was longer than that of the latter.

The depressive effect of CRD-401 on ouabain arrhythmias appears inferior to that of propranolol when the antagonists were administered before the onset of arrhythmias, but both drugs showed similar effects when administered during development of various arrhythmias.

Quinidine had little or no effect against epinephrine and ouabain arrhythmias in doses of 1.0 to 2.0 mg/kg.

The $\beta$-blocking action of propranolol was competitive in accordance with the result obtained by Black et al. (8), while CRD-401 depressed the maximal response to the $\beta$-agonist, indicating a non-competitive antagonism (10).

The antiarrhythmic effect of CRD-401 is considered to be related to the membrane stabilizing effect (M. Hoshiyama, personal communication).
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