Cardiovascular Effects of 2-amino-N-(2,6-dimethylphenyl)-N-[3-(3-pyridyl)propyl]propionamide dihydrochloride (Ro 22-9194) in the Isolated, Cross-Perfused Atrium of the Dog

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SUMMARY

The cardiovascular effects of 2-amino-N-(2,6-dimethylphenyl)-N-[3-(3-pyridyl)propyl]propionamide dihydrochloride (Ro 22-9194) were investigated in the canine isolated atrial preparation perfused with arterial blood from another donor dog. Intravenous administration of Ro 22-9194 (0.3-3 mg/kg) reduced heart rate and systemic arterial blood pressure of the donor dog. Simultaneously, in the isolated atrium perfused with donor’s blood, negative chronotropic and inotropic responses were induced in a dose-dependent manner. Direct administration of Ro 22-9194 (1-300 µg) into the sinus node artery of the isolated atrium induced negative chrono- and inotropic responses and a transient increase in coronary blood flow in a dose-related manner. Mexiletine (1-300 µg) also induced negative chrono- and inotropic responses. The potency of Ro 22-9194 for negative cardiac effects was similar to that of mexiletine. The negative cardiac effects of Ro 22-9194 were not modified by atropine in doses which significantly inhibited the cardiac effects of acetylcholine. Ro 22-9194 did not affect the cardiac responses to norepinephrine and to acetylcholine significantly. These results suggest that Ro 22-9194 has non-cholinergic cardiac depressant properties with its vasodilating action, and it has neither anti-adrenergic nor anti-cholinergic actions on the dog heart.

Additional Indexing Words:
Ro 22-9194 Isolated dog atrium SA node Pacemaker activity Atrial contractility

RECENTLY, a new compound, 2-amino-N-(2,6-dimethylphenyl)-N-[3-(3-pyridyl)propyl]propionamide dihydrochloride (Ro 22-9194), having a
chemical structure similar to lidocaine, was synthesized for its predominant antiarrhythmic properties. Lidocaine and mexiletine are useful for treating ventricular arrhythmias in experimental models and clinical situations. They are classified as class I antiarrhythmic drugs on the classification of Vaughan Williams. Class I antiarrhythmic drugs share the ability to block the fast inward sodium current in cardiac muscles. In addition, some class I antiarrhythmic drugs influence the autonomic nervous system, and in particular, have anti-cholinergic activity. In the present experiments, therefore, we studied the cardiovascular effects of Ro 22-9194 in the canine isolated, blood-perfused atrial preparation, and its effects on the cardiac responses to norepinephrine or acetylcholine.

**Methods**

Isolated, blood-perfused atrial preparations:

A canine isolated right atrium was perfused with heparinized arterial blood from a donor dog. The details of the preparation have been described in previous papers.

Right atrial preparations were obtained from 11 dogs weighing 8–16 kg. Each dog was anesthetized with sodium pentobarbital (30 mg/kg i.v.). After treatment with sodium heparin (200 USP U/kg i.v.), the right atrium was excised and immersed in cold Ringer’s solution. The sinus node artery was cannulated via the right coronary artery and was perfused with heparinized blood led from the carotid artery of the donor dog with the aid of a peristaltic pump (Harvard Apparatus, model 1210). A pneumatic resistance was placed in parallel with the perfusion system so that the perfusion pressure could be maintained at 100 mmHg. The venous effluent from the preparation was led to a collecting funnel, from which it was returned continuously to the donor dog via an external jugular vein. The ventricular margin of the atrium was attached to a rigid stainless steel bar, and the preparation was placed in a glass container which was kept at a constant temperature of 37°C by means of a heating bath circulator (Haake, FE 2). The superior part of the atrium was connected to a force-displacement transducer (Nihon Kohden, AP 620G) by a silk thread. The atrial muscle was usually stretched to a resting tension of 2 g. The isometric tension was recorded on a thermo-writing rectigraph (Nihon Kohden, WT 685T). A pair of silver electrodes was brought into contact with the epicardial surface to record the atrial electrogram from which the sinus rate was derived with a cardiotachometer (Nihon Kohden, AT 600G). The blood flow rate to the preparation was recorded by means of a magnetic blood flow-meter (Nihon Kohden, MFV 2100). The
rate of blood flow to the isolated atrium was 3 to 12 ml/min.

Eleven donor dogs weighing 10-25 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and supplemental doses of sodium pentobarbital were applied as necessary to maintain stable anesthesia. Dogs were artificially ventilated with room air by use of a Harvard respirator (model 607). Sodium heparin (500 USP U/kg i.v.) was administered at the beginning of the perfusion of the isolated atrial preparation, and 200 USP U/kg given each hour thereafter. The femoral arterial blood pressure and heart rate derived from lead II of the ECG of the donor dog were recorded simultaneously.

**Drugs:**

Drugs used in this study were 2-amino-N-(2,6-dimethylphenyl)-N-[3-(3-pyridyl)propyl]proponamide dihydrochloride (Ro 22-9194; Kissei Pharmaceutical Co., Fig. 1), mexiletine hydrochloride (extracted from Mexitil; Dainippon), acetylcholine chloride (Daiichi), atropine sulfate (Takeda) and nor-epinephrine hydrochloride (Sankyo). Drugs were injected into the sinus node artery of the isolated right atrium through a rubber tube with a microsyringe (Terumo). The amount of drug solution was 0.01 to 0.03 ml and it was injected over a period of 4 sec. Ro 22-9194 was also administered intravenously via the jugular vein of the donor dog.

**Statistical analysis:**

Data are shown as the maximum change in response to each drug, and expressed as mean±SEM. Student's t-test for paired or unpaired data was carried out in comparisons between 2 groups. A p value less than 0.05 was considered as statistically significant.

![Chemical structure of Ro 22-9194](image-url)
RESULTS

Effects of intravenous administration of Ro 22-9194 on donor dogs and isolated atria:

Ro 22-9194 (1 and 3 mg/kg) injected into the jugular vein of a donor dog decreased heart rate and arterial blood pressure in a dose-dependent manner as shown in Fig. 2A. One and one half min after administration of the drug, negative chronotropic and inotropic responses were induced in an isolated atrium perfused with arterial blood from a donor dog (Fig. 2B). These cardiovascular responses to intravenous injections of Ro 22-9194 at doses of 0.3, 1 and 3 mg/kg are summarized in Fig. 3.

Effects of Ro 22-9194 on SA nodal pacemaker activity and atrial contractility of the isolated atrium:

When Ro 22-9194 at doses of 1–300 µg was directly injected into the sinus node artery of an isolated atrium, negative chronotropic and inotropic effects were induced in a dose-dependent manner as shown in Fig. 4. Ro 22-9194 transiently increased the blood flow rate to the isolated atrium. Figure 5 shows the effects of mexiletine, a class Ib antiarrhythmic drug, injected into the sinus node artery of an isolated atrium. Mexiletine at doses of 1–300 µg decreased the sinus rate and atrial contractility in a dose-dependent manner. The negative chronotropic and inotropic responses to Ro 22-9194 and mexiletine are summarized in Fig. 6. The dose-response curves are also shown for the negative chronotropic and inotropic effects of lidocaine and phenytoin in the isolated atrial preparation cited from a previous paper by Chiba.9)

Fig. 2. Typical tracings of the effects of Ro 22-9194 on heart rate and blood pressure of a donor dog (A) and on sinus rate and atrial tension of a canine isolated atrium perfused with donor's blood (B). Ro 22-9194 (1 and 3 mg/kg) was administered intravenously to a donor dog.
Fig. 3. Effects of intravenous administration of Ro 22-9194 on heart rate and mean blood pressure of the donor dog (A) and on sinus rate and atrial tension of the canine isolated atrium perfused with donor's blood (B). The basal heart rate and mean blood pressure in 5 donor dogs were 161±14 (mean±SEM) beats/min and 75±3 mmHg, respectively, and the basal sinus rate and atrial tension in 5 isolated atria were 115±8 beats/min and 1.7±0.3 g, respectively.

Effects of atropine on negative chronotropic and inotropic responses to Ro 22-9194:
The negative chronotropic and inotropic responses to Ro 22-9194 (100 µg) were not inhibited by the dose of atropine (3 µg) that abolished the negative chronotropic and inotropic responses to acetylcholine (0.1 µg) in an isolated atrium (Fig. 7). These results were confirmed in 4 experiments.

Fig. 4. A typical tracing of the effects of Ro 22-9194 injected into the sinus node artery on sinus rate and atrial tension in a canine isolated, blood-perfused atrium.
Fig. 5. A typical tracing of the effects of mexiletine injected into the sinus node artery on sinus rate and atrial tension in a canine isolated, blood-perfused atrium.

Fig. 6. Dose-response curves for negative chronotropic and inotropic responses to Ro 22-9194 and mexiletine injected into the sinus node artery of the canine isolated, blood-perfused atrium. The basal sinus rate and atrial tension in 6 experiments were 116 ± 10 (mean ± SEM) beats/min and 2.8 ± 0.4 g, respectively. Dose-response curves of negative chronotropic and inotropic responses for lidocaine and phenytoin are adapted from a previous paper of Chiba.9)

Effects of Ro 22-9194 on cardiac responses to norepinephrine or acetylcholine:
Ro 22-9194 (100 or 300 µg) did not affect norepinephrine (0.01 or 0.03 µg)-induced positive chronotropic and inotropic responses in 5 isolated atria (Table I). Ro 22-9194 did not modify the negative chronotropic and
Fig. 7. A typical tracing of the effects of atropine on Ro 22-9194- and acetylcholine (ACh)-induced negative chronotropic and inotropic responses in a canine isolated, blood-perfused atrium.

Table I. Effect of Ro 22-9194 (100-300 μg) on Chronotropic and Inotropic Responses Induced by Norepinephrine (0.01-0.03 μg) or Acetylcholine (0.03-0.3 μg) in the Canine Isolated, Blood-Perfused Atrium

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>After Ro 22-9194 treatment</th>
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<tbody>
<tr>
<td></td>
<td>SR (%)</td>
<td>AT (%)</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>16.3±3.7</td>
<td>14.8±2.8</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>−33.0±12.9</td>
<td>−22.4±14.1</td>
</tr>
</tbody>
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Each value indicates the mean±SEM.
SR=sinus rate; AT=atrial tension.
The basal sinus rate and atrial tension in 5 experiments were 116±10 beats/min and 2.9±0.5 g, respectively.

The effects of Ro 22-9194 and acetylcholine (ACh) on heart rate and systemic arterial blood pressure after intravenous administration to the donor dog. In the isolated atrial preparation perfused with arterial blood from the donor dog, Ro 22-9194 injected intravenously into the donor dog or into the sinus node artery of the isolated atrium decreased sinus rate and atrial contractility in a dose-dependent manner. The negative chronotropic and inotropic responses to Ro 22-9194 were not affected by an adequate dose of atropine. Ro 22-9194 did not modify the positive cardiac responses to norepinephrine or the negative cardiac responses to acetylcholine. These re-
suits indicate that Ro 22-9194 has direct cardiac depressant actions with vasodilation but has neither anti-adrenergic nor anti-cholinergic actions on the dog heart.

The chemical structure of Ro 22-9194 is similar to that of lidocaine which is classified as a class Ib antiarrhythmic drug. In the canine isolated, blood-perfused atrial preparation, we have analyzed the cardiac effects of antiarrhythmic drugs of each class in the present and previous studies. For class Ib antiarrhythmic drugs and Ro 22-9194, the order of potencies for inducing a negative chronotropic effect was lidocaine > mexiletine > Ro 22-9194 > phenytoin (Fig. 6, upper panel). On the other hand, the order of potencies for inducing a negative inotropic effect was lidocaine > phenytoin > mexiletine > Ro 22-9194 (Fig. 6, lower panel). Thus, in the dog heart Ro 22-9194 has mild negative chronotropic and inotropic effects in comparison with class Ib antiarrhythmic drugs. However, Ro 22-9194-induced negative cardiac responses tended to last longer than those induced by either mexiletine (Figs. 4 and 5) or lidocaine.

Ro 22-9194 in a fashion similar to lidocaine and mexiletine transiently induced vasodilation, i.e., Ro 22-9194 increased the blood flow rate to the sinus node artery of the isolated atrium and decreased arterial blood pressure of the donor dog after intravenous administration (Figs. 2 and 3).

The negative chronotropic and inotropic responses to Ro 22-9194 were not blocked by atropine in doses which abolished the negative cardiac responses to acetylcholine, indicating that the negative cardiac responses to Ro 22-9194 are not mediated by the muscarinic receptors. Honerjager et al have concluded that the negative inotropic effects of class I antiarrhythmic drugs were the sum of their sodium channel blocking and some additional drug-dependent inotropic properties.

Ro 22-9194 did not inhibit either positive cardiac responses to norepinephrine or negative cardiac responses to acetylcholine in the isolated atrium of the dog. Antiarrhythmic drugs at higher doses mostly have anti-adrenergic or/and anti-cholinergic properties. Lidocaine and phenytoin, class Ib antiarrhythmic drugs, in addition to the class Ia drugs quinidine and procainamide, have anti-cholinergic properties in the canine isolated, blood-perfused atrial preparation and other heart preparations. However, Ro 22-9194 doses used in the present study did not depress the negative chronotropic and inotropic responses to acetylcholine. In the isolated, blood-perfused dog atrium, phenytoin at extremely high doses depressed the positive inotropic effect of norepinephrine, and amiodarone depressed the positive chronotropic effect of norepinephrine. Thus, it is concluded that Ro 22-9194 at the doses used in the present study has neither anti-cholinergic nor anti-adrenergic pro-
properties on the dog heart.

REFERENCES