PHARMACOLOGIC ANALYSIS OF CHRONOTROPIC AND INOTROPIC RESPONSES TO 5-HYDROXYTRYPTAMINE IN THE DOG HEART

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Abstract—Inotropic and chronotropic effects of 5-hydroxytryptamine (5-HT) were investigated in twenty-five isolated canine atrial preparations and three isolated paced ventricular preparations which were suspended in the bath and perfused with arterial blood from the carotid artery of the heparinized support dog. 5-HT was administered into the cannulated sinus node artery in a dose range of 1 to 300 μg. In small doses of 1 to 3 μg, 5-HT induced a negative chronotropic and inotropic response. At relatively higher doses over 10 μg, 5-HT induced a biphasic response: a slight negative chronotropic and inotropic response, followed by a long-lasting positive response. With large doses, only a positive chronotropic and inotropic response was induced in the majority of cases. Even in the ventricular preparations, 5-HT produced a similar response pattern in inotropism. 5-HT-induced positive chronotropic and inotropic responses were completely inhibited by pretreatment with an adrenergic beta-blocking agent, propranolol, or a non-depressant beta-blocking agent, carteolol, and desipramine, but were not influenced by tetrodotoxin treatment. After the treatment with carteolol or despramine, 5-HT-induced negative responses were potentiated. 5-HT-induced negative chronotropic and inotropic responses were not inhibited by methysergide and atropine. From these results, it is suggested that 5-HT induces a direct negative chronotropic and inotropic response and an indirect positive response via its tyramine-like action.

5-Hydroxytryptamine (5-HT, serotonin) has positive inotropic and chronotropic effects of varying degrees on isolated hearts, atria and isolated papillary muscles of various species (1, 2). In 1964, James (3) reported that 5-HT had only a negative chronotropic effect on the canine sino-atrial (SA) node in vivo, in contrast to its reported acceleratory action in isolated heart preparations. In 1967, Buccino et al. (4) studied effects of 5-HT in cut papillary muscles and canine right-heart-bypass preparations with bilateral carotid sinus and vagal nerve resection. They reported that 5-HT increased the average peak tension of cat papillary muscles or dog left ventricles and concluded that 5-HT has a direct positive inotropic effect on mammalian myocardium. Such a postulation was based on the findings that the augmentation in either rate of tension developed or peak isometric tension produced by 5-HT was not accompanied by changes in time to peak tension, differing from actions of norepinephrine (5, 6). However, they did not use pharmacological drugs for the analysis of effects of 5-HT. In 1971, Fillion et al. (7) reported that when using biochemical assay techniques, release of norepinephrine from the dog heart in situ occurred after intravenous and intracoronary administration of 5-HT.

In the present study, effects of 5-HT on pacemaker activity and contractility were in-
vestigated using the isolated, blood-perfused canine atrial preparation developed by Chiba et al. (8, 9) in 1975. Moreover, the isolated, blood-perfused ventricular preparation developed by Chiba (10) in 1976 was also employed in three experiments.

MATERIALS AND METHODS

Twenty-five adult mongrel dogs of either sex weighing 11-16 kg were anesthetized with sodium pentobarbital, 30 mg/kg, i.v.. The right atrium or left ventricle was quickly excised and immersed in Tyrode solution at about 4-10°C. The isolated right atrial muscle was perfused with arterial blood through the cannulated sinus node artery, and the isolated left ventricular muscle was perfused through the cannulated anterior descending branch of the left coronary artery. The blood was fed from the carotid artery of the heparinized support dog under the constant pressure of 100 mm Hg by aid of a peristaltic pump (Harvard Apparatus 1210). The atrium or the ventricle was suspended in a bath filled with blood at a constant temperature of 37°C. Sinus rate was recorded with a tachometer (Nihon Kohden RT-5) which was triggered by the atrial action potentials, and isometric tension development was measured with a force displacement transducer (Grass FTO3B). The ventricular muscle was electrically driven with rectangular pulses by use of an electronic stimulator (Nihon Kohden MSE-3). The stimulus strength was 5 msec and 0.1-1 volts, about twice the threshold voltage, at 1.5-2 Hz. Details of the isolated, blood-perfused canine atrium or ventricular preparation are described in previous papers (8-10). The volume of drug solution injected with microinjectors was 0.01-0.03 ml in a period of 4 sec.

Drugs used in this study were serotonin creatinine sulfate (5-hydroxytryptamine, 5-HT) (Sandoz Ltd, Basel), (±)-propranolol hydrochloride (Sumitomo Chemicals), (±)-norepinephrine hydrochloride (Sankyo), (±)-carteolol hydrochloride ([±]-5(3-tert-butylamino-2-hydroxy)propoxy-3,4-dihydrocarbostyril hydrochloride) (Otsuka Pharm. Co.), tetrodotoxin (Sankyo), nicotine (base), tyramine hydrochloride (Waco), desipramine (Fujisawa), acetylcholine chloride (Daiichi) and atropine sulfate (Takeda).

RESULTS

Effects of 5-HT on isolated, blood-perfused atrial preparations

5-HT was administered into the cannulated sinus node artery. A relatively small dose of 5-HT, 1-3 µg, produced a monophasic slight negative chronotropic and inotropic effect. A relatively higher dose, about 10 µg, induced a biphasic response: a primary negative chronotropic and inotropic response, followed by a long-lasting positive response. In this case, a negative inotropic response was not so clear as the negative chronotropic response. A higher dose produced only a positive response in the majority of cases. Fig. 1 shows records from an experiment with increasing doses of 5-HT. Results are summarized in Table 1.

Effect of a beta-adrenoceptor blocking agent, propranolol or a non-depressant beta-adrenoceptor blocking agent, carteolol, on the responses to 5-HT

When propranolol was injected into the sinus node artery, negative chronotropic and
FIG. 1. Chronotropic and inotropic responses to increasing doses of 5-hydroxytryptamine (5-HT) injected into the cannulated sinus node artery of an isolated canine atrium.

FIG. 2. Effect of 3 µg of propranolol on the responses to 0.03 µg of norepinephrine (NE) and 100 µg of 5-HT in an isolated canine atrium.

inotropic effects were usually produced. On the other hand, the administration of carteolol, a more potent adrenergic beta-blocking agent, caused positive chronotropic and inotropic effects. The positive chronotropic and inotropic responses to norepinephrine and 5-HT were completely blocked by the pretreatment of tissue with either propranolol or carteolol. Fig. 2 shows that propranolol inhibited norepinephrine- and 5-HT-induced positive chrono-
Effects of carteolol on the 5-HT-induced chronotropic and inotropic responses

When carteolol was injected into the sinus node artery, 5-HT-induced positive chronotropic and inotropic effects were completely inhibited. 5-HT-induced negative chronotropic and inotropic responses were to some extent enhanced as shown in Fig. 3 and Table 2.

**Effects of desipramine on the 5-HT- and tyramine-induced chronotropic and inotropic responses**

When desipramine was injected into the sinus node artery, long-lasting positive chronotropic and inotropic effects following brief negative chronotropic and inotropic effects usually occurred. After treatment of tissue with desipramine, tyramine- and 5-HT-induced positive chronotropic and inotropic effects were significantly suppressed, although norepinephrine-induced positive effects were not suppressed as shown in Fig. 4 and Table 3. 5-HT frequently caused profound negative chronotropic and inotropic effects after desipramine treatment.

**Absence of blocking effect of tetrodotoxin on the 5-HT-induced positive chronotropic and inotropic actions**

When nicotine, 1-10 μg, was injected into the sinus node artery, biphasic chronotropic and inotropic responses were observed. 5-HT-induced positive responses were completely inhibited by carteolol, and 5-HT-induced negative chronotropic and inotropic responses were to some extent enhanced as shown in Fig. 3 and Table 2.
FIG. 4. Effects of 100 μg of desipramine (DMI) on the responses to 0.03 μg of norepinephrine (NE), 1 μg of tyramine and 300 μg of 5-HT in an isolated canine atrium.

FIG. 4. Effects of 100 μg of desipramine (DMI) on the responses to 0.03 μg of norepinephrine (NE), 1 μg of tyramine and 300 μg of 5-HT in an isolated canine atrium.

Table 3. Effect of desipramine on the 5-HT and tyramine-induced chronotropic and inotropic response

<table>
<thead>
<tr>
<th>Dose of drugs (μg)</th>
<th>No. of expts.</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NCE (%)</td>
<td>PCE (%)</td>
</tr>
<tr>
<td>5-HT 30-100</td>
<td>6</td>
<td>1±0.4</td>
<td>12±2.8</td>
</tr>
<tr>
<td>Tyramine 1</td>
<td>6</td>
<td>—</td>
<td>14±3.5</td>
</tr>
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</table>

Significantly different from respective controls by t-test (*: P<0.05, **P<0.01). See Table 2 for abbreviations. The mean control sinus rate was 103±4.2 beats/min in 6 preparations.

and inotropic effects were produced. These nicotine-induced effects were significantly suppressed by pretreatment with tetrodotoxin, but tyramine-induced effects were not so influenced as reported previously (11, 12). Effects of 30-100 μg of 5-HT were not affected by 3-10 μg of tetrodotoxin in 2 preparations, although effects of 1-10 μg of nicotine were clearly suppressed.

Absence of blocking effect of atropine or methysergide on the negative chronotropic and inotropic responses to 5-HT

When acetylcholine was injected into the sinus node artery, a negative chronotropic and inotropic response was produced. The negative chronotropic and inotropic responses to 5-HT were not influenced by pretreatment of the tissue with atropine (10-30 μg) which antagonized responses to 0.1-0.3 μg of acetylcholine (Table 4A).

When methysergide was injected into the sinus node artery, negative chronotropic and inotropic effects were usually induced. 5-HT-induced negative responses were not significantly suppressed by treatment with methysergide (Table 4B).
TABLE 4. Effect of atropine (A) and methysergide (B) on the negative chronotropic and inotropic effects of 5-HT in isolated atrium preparation of dog

(A)

<table>
<thead>
<tr>
<th>Dose of drugs (µg)</th>
<th>No. of expts.</th>
<th>Atropine treatment (10–30 µg)</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>NCE (%)</td>
<td>NIE (%)</td>
<td>NCE (%)</td>
</tr>
<tr>
<td>ACh 0.1–0.3</td>
<td>3s</td>
<td>32±13.6</td>
<td>37±11.9</td>
<td>0*</td>
</tr>
<tr>
<td>5-HT 1–3</td>
<td>3</td>
<td>4±0.7</td>
<td>5±2.9</td>
<td>4±0.7</td>
</tr>
</tbody>
</table>

(B)

<table>
<thead>
<tr>
<th>Dose of 5-HT (µg)</th>
<th>No. of expts.</th>
<th>Methysergide treatment (10–30 µg)</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NCE (%)</td>
<td>NIE (%)</td>
<td>NCE (%)</td>
</tr>
<tr>
<td>1</td>
<td>5s</td>
<td>4.2±0.4</td>
<td>5.8±1.7</td>
<td>3.8±0.4</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>6.0±1.2</td>
<td>9.0±2.1</td>
<td>4.7±1.8</td>
</tr>
</tbody>
</table>

Significantly different from respective controls by t-test (*: P<0.05). See Table 2 for abbreviations. a: the mean control sinus rate was 103±3.3 beats/min in 3 preparations. b: the mean control sinus rate was 91±8.3 beats/min in 5 preparations.

Effects of 5-HT on the isolated ventricle paced electrically

When 5-HT was injected into the cannulated anterior descending branch of the isolated left ventricular muscle paced electrically at 1.5–2 Hz, a positive inotropic response was dose-relatedly induced with or without a brief and slight negative inotropic response in all three preparations. Fig. 5 shows the results of an experiment on a ventricular muscle preparation when increasing doses of 5-HT were applied. These positive inotropic effects of 5-HT were completely blocked by treatment with 10 µg of propranolol or 1 µg of carteolol.

DISCUSSION

The present experiments demonstrate that intra-arterial injections of 5-HT produce direct negative chronotropic and inotropic effects and indirect positive chronotropic and inotropic effects in the blood-perfused atrium and ventricular preparation of dogs. The
negative inotropic effects were less marked than the negative chronotropic effects to 5-HT. 5-HT induced negative chronotropic and inotropic effects were usually not so prominent regardless of the dose. This is in part due to counteraction by indirect adrenergic effects induced by 5-HT, because the negative effects of 5-HT were quite evident after treatment of tissues with desipramine or, a non-depressant beta-adrenoceptor blocking agent, carteolol. Since 5-HT-induced negative effects were not modified by pretreatment of the tissues with dose levels of atropine sufficient to block muscarinic receptors, cholinergic mechanisms are apparently not involved in the response to 5-HT in the isolated preparations. Previously, James (3) reported that methysergide did not block the 5-HT-induced negative chronotropic effect as determined with a direct perfusion method of the sinus node artery in vivo. Even in the present study, there was no evidence of antagonism of 5-HT by methysergide in the SA node. It is well known that in a variety of vascular smooth muscle, methysergide inhibits the vasoconstrictor and pressor effects of 5-HT as well as the action of the amine (2). Therefore, the cardiac 5-HT receptor probably differs from extracardiac 5-HT receptors.

In the dog, rapid intravenous injection of 5-HT produces initial bradycardia followed by marked tachycardia (13) or sinus tachycardia (14). Heart contractile force is increased, at least initially (13, 15). In 1964, James (3) reported that 5-HT has only a negative chronotropic effect on the sinus node in vivo. Nevertheless, in the present study using isolated preparations, 5-HT induced dose-relatedly positive chronotropic and inotropic effects with or without slight negative effects. The discrepancy may in part be the result of different procedures. James interrupted the perfusion flow by use of a three-way stopcock when the drug solution was injected at a volume of 1 ml into the sinus node artery. Accordingly, even when Ringer's solution was injected, marked changes in heart rate were always induced (16). These changes in heart rate may thus have been due to changes in the perfusion pressure. On the contrary, in the present study, a constant pressure perfusion system was arranged by means of a Starling resistance as reported previously, and moreover, the volume of the injected drug solution was so small (0.01-0.03 ml) that changes in heart rate were not significant even with Ringer's solution (17, 18). Thus, the perfusion flow was never interrupted during the experiment. Since the SA node is most sensitive to changes in perfusion pressure of the sinus node artery, a constant perfusion system provides the best convenience to observe chronotropic responses to a drug. Another difference may be partially due to different control sinus rates of the preparations. In the study of in vivo direct perfusion of the SA node by James (3), the control sinus rate was 126 to 175 beats/min in all ten preparations where sodium pentobarbital had served as the anesthesia, while in the present work using isolated preparations, the control sinus rate was approximately 100 beats/min in all preparations. Therefore, in in vivo preparations, a relatively higher sinus rate which may be produced by tonic sympathetic tone may influence the appearance of the positive effect of 5-HT.

However, it has been established that the cardiac stimulatory effects of 5-HT are mediated by catecholamines (7, 19-22). In the present study, it was confirmed that 5-HT produced
dose-relatedly positive chronotropic and inotropic effects through an adrenergic mechanism. The 5-HT-induced positive effects as well as norepinephrine-induced ones were completely blocked by beta-adrenoceptor blocking agents, not only propranolol, but also carteolol which is reported by Yabuuchi and Kinoshita to be a non-depressant (23). Furthermore, the responses to 5-HT were not inhibited by treatment with tetrodotoxin but were suppressed by desipramine. Thus, in the blood-perfused atrium preparations, the positive chronotropic and inotropic responses to 5-HT may be due to indirect catecholamine release via its tyramine-like action. In the canine ventricle, Buccino et al. (4) reported that 5-HT exerts a direct positive inotropic effect. However, they did not use beta-adrenoceptor blocking agents. In the present study of the isolated left ventricular preparations, a positive inotropic effect of 5-HT was apparently due to an indirect effect via an adrenergic mechanism, as it was completely inhibited by beta-adrenoceptor blocking agents.

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