The Effect of 5-Hydroxytryptamine on the Slow Inward Current of Bullfrog Atrium

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SUMMARY

The effect of 5-hydroxytryptamine (5-HT) on the isometric contraction, membrane potential, and membrane current of isolated bullfrog (Rana catesbeiana) atrium was investigated. 5-HT depressed the isometric contraction and this negative inotropism was atropine-resistant.

5-HT reduced both peak amplitude and duration of the action potential, but caused no detectable changes in the resting membrane potential and membrane input resistance. The final, or "secondary depolarization phase" in the rising phase of the action potential was found to be selectively depressed by 5-HT. These inhibitory effects on the action potential were also atropine-resistant.

A single sucrose-gap voltage-clamp experiment revealed that 5-HT caused a reduction of the slow inward (Ca++/Na+) current. The membrane slope conductance near the resting membrane potential and the degree of activation of the time-dependent potassium current showed no detectable change in the presence of 5-HT.

It was concluded on the basis of the present results that 5-HT directly controlled the action potential by selectively depressing the slow inward (Ca++/Na+) current. This may be responsible for the negative inotropic effect of 5-HT on bullfrog atrium.

Additional Indexing Words:
Electrophysiology  5-HT  Negative inotropy  Calcium current

The effects of 5-HT on the mechanical response of heart muscles have been studied on mammalian heart, and it is suggested that 5-HT has an initial negative inotropic and chronotropic effects followed by positive inotropic and chronotropic effects. The stimulant effect of 5-HT was reported to be the effect of noradrenaline released by 5-HT from sympathetic nerve terminals in the heart. However, the mechanism which is responsible for the initial negative inotropic and chronotropic effect is not clarified yet.

It was demonstrated that the duration of action potential of frog ventricle was shortened by the effect of 5-HT. On the other hand, a voltage-clamp experiment revealed that 5-HT reduced the inward Ca++ current in

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the action potential of embryonic chick sensory neurons.\textsuperscript{10} It may be therefore worthwhile to examine the possibility that 5-HT directly controls the Ca\textsuperscript{++} current during the generation of action potential. Present results showed that the depression of slow inward (Ca\textsuperscript{++}/Na\textsuperscript{+}) current under the effect of 5-HT was involved in the mechanism for the reduction of mechanical response of bullfrog atrium.

**Materials and Methods**

Strips (0.3–0.5 × 3 mm in size) of quiescent muscle fibre bundles excised from the atrium of bullfrog (\textit{Rana catesbeiana}) heart were used. A single sucrose-gap voltage-clamp apparatus including a chamber for mounting preparation was described elsewhere,\textsuperscript{11} and was essentially similar to that reported by Beeler and Reuter.\textsuperscript{12} Action potentials were induced by applying electrical pulse stimulations (0.1–0.2 Hz) across the sucrose-gap or simply through a pair of platinum electrodes which were placed very close to the strip and they were simply recorded by an intracellular microelectrode filled with 3 M KCl.

Experiment which was designed to study the isometric contraction of atrial muscle was performed by larger strips (2 × 7 mm in size). A force-displacement transducer was used to record the isometric contraction of atrial muscle. Contractions were elicited by electrical pulses (1 msec in duration) through a pair of platinum electrodes placed very close to the strip at the frequency of 0.2–0.3 Hz.

Ionic compositions of solutions used in the present experiment were as follows; Sucrose solution, 240 mM sucrose. Ringer solution; 112 mM NaCl, 2 mM KCl, 1.8 mM CaCl\textsubscript{2}, 2.4 mM NaHCO\textsubscript{3}, and 2.5 mM glucose. Ringer solution was oxygenated by 95% O\textsubscript{2} and 5% CO\textsubscript{2}.

Drugs added to Ringer solution were as follows; 5-hydroxytryptamine creatinine sulfate (Wako Pure Chem. Ind.) acetylcholine chloride (Wako Pure Chem. Ind.), atropine sulfate (MERCK) and creatinine sulfate (SIGMA). The preparation was continuously perfused with Ringer solution, and all experiments were carried out at room temperature (18–22°C).

According to the preliminary experiment, it was previously confirmed that creatine-sulfate (5 × 10\textsuperscript{-5} M) had no detectable effect on the slow inward current of the atrium.

**Results**

1. Effect of 5-HT on isometric contractions

The isometric contraction of bullfrog atrium was measured under the effect of 5-HT in various concentrations. 5-HT consistently caused a negative inotropic effect when it was applied for short period. Fig. 1 shows one of the typical results of these experiments. In this experiment, after control contractions were obtained in Ringer solution, perfusate was switched to Ringer solution containing 2 × 10\textsuperscript{-5} M of 5-HT. As seen in this figure, iso-
metric contraction of atrial muscle was markedly depressed by 5-HT; the negative inotropy appeared within 30 sec and reached to the maximum within 2–3 min after its application. After 5-HT was washed out, isometric contractions reversibly recovered to the control within 10–15 min. The negative inotropy was observed to be dose-dependent; the effective minimum concentration was $5 \times 10^{-7}$ M. No positive inotropy was observed during such a short period of application of 5-HT.

Fig. 2. Effect of atropine on the 5-HT induced negative inotropic effect. A: Isometric contractions in ACh ($5 \times 10^{-8}$ M) and 5-HT ($5 \times 10^{-5}$ M) with and without atropine ($3 \times 10^{-8}$ M). B: Changes in the amplitude of isometric contraction were plotted against time. Amplitude in Ringer solution was taken as 100%. The protocol of drug application was illustrated in upper part of the graph.
2. Effect of atropine on the 5-HT induced negative inotropic effect

The negative inotropic effect of 5-HT was examined in the presence of atropine. As seen in Fig. 2, acetylcholine (ACh) \( (5 \times 10^{-8} \text{ M}) \) and 5-HT \( (5 \times 10^{-5} \text{ M}) \) showed comparable inhibitory effects on the isometric contraction. In the presence of a relatively low concentration of atropine \( (3 \times 10^{-8} \text{ M}) \), the effect of ACh was completely antagonized, whereas that of 5-HT remained almost unaffected.

3. Effect of 5-HT on action potentials

Action potentials of bullfrog atrium were studied in the presence of 5-HT at various concentrations. Fig. 3 shows one of the typical results obtained from the experiment which was designed to study the effect of 5-HT \( (5 \times 10^{-5} \text{ M}) \) on the action potential. As seen in Fig. 3-A, 5-HT caused a marked shortening of the action potential and a significant decrease in its peak amplitude, but caused no detectable changes in resting membrane potential. Fig. 3-B illustrates the initial depolarizing phase of the action potentials which are shown in Fig. 3-A. The final, or "secondary depolarization phase" \( ^{11} \) of the action potential was selectively reduced by this dose of 5-HT (Fig. 3-B-b). The inhibitory effect of 5-HT was reversible. No stimulant responses were observed during such a short period of application of 5-HT. The effect of \( 5 \times 10^{-5} \text{ M} \) of 5-HT on action potential amplitude (AP\text{Amp}), action potential duration at 20% height of the control action potential (APD 20%), and APD 80% were 93.9±0.8%, 79.1±3.0%, and 64.4±3.1% (mean±SE, n=6), respectively. Inhibitory effects were con-
sistent observed in dose-dependent manner with concentrations between 5-100×10⁻⁶ M.

4. Effect of 5-HT on the action potential in the presence of atropine

Effect of 5-HT on the action potential was investigated in Ringer solution containing a relatively low concentration (3×10⁻⁸ M) of atropine. Atropine in this concentration had no effect on action potentials but completely antagonized the muscarinic effect of ACh (1×10⁻⁷ M). Fig. 4 shows a typical result of the experiment which was designed to demonstrate the direct effect of 5-HT (5×10⁻⁵ M) on the action potential; changes in the peak amplitude of action potentials were plotted in Fig. 4-B. As demonstrated in Fig. 4-A, ACh (1×10⁻⁷ M) and 5-HT (5×10⁻⁵ M) suppressed both peak amplitude and duration of the action potential. Atropine completely antagonized the effect of ACh but could not that of 5-HT. In 3 preparations, the effect of 5×10⁻⁵ M of 5-HT under the presence of atropine (3×10⁻⁸ M) on APAm, APD 20%, and APD 80% were 92.0±1.2%, 85.7±0.9%, and 70.0±2.5% (mean±SE, n=3), respectively. These results

![Diagram](image-url)

Fig. 4. Effect of 5-HT on the action potential in the presence of atropine. A: Action potentials in ACh (1×10⁻⁷ M) and 5-HT (5×10⁻⁵ M) with and without atropine (3×10⁻⁸ M). B: Changes in peak amplitude of action potentials were plotted against time. Peak amplitude in Ringer solution was taken as 100%. The protocol of drug application was illustrated in upper part of the graph. Alphabetical marks correspond to those in A.
clearly indicated that 5-HT directly affected on bullfrog atrium and suppressed the action potential.

5. Effect of 5-HT on the slow inward (Ca\(^{++}\)/Na\(^{+}\)) current

The effect of 5-HT on the slow inward (Ca\(^{++}\)/Na\(^{+}\)) current (\(I_{\text{sl}}\)) was studied. Fig. 5 shows one of the results of the voltage-clamp experiment (6 preparations) which was designed to investigate the effect of 5-HT on \(I_{\text{sl}}\) of the atrium. These results are summarized in Table I. The preparation (No. 1, Table I) was perfused with Ringer solution, and 700 msec rectangular voltage-clamp pulses were applied from the holding potential \((-42 \text{ mV})\) to \(-12 \text{ mV}\) at the frequency of approximately 0.1 Hz. According to such an experimental procedure, \(I_{\text{sl}}\) which was TTX insensitive and Mn\(^{++}\) sensitive was observed. The fast sodium channel was inactivated by holding the membrane potential at \(-42 \text{ mV}\) from the resting potential \((-90 \text{ mV})\).\(^{12}\)

Table I. The Effect of 5-HT (5\(\times 10^{-5}\)M) on the Slow Inward Current

<table>
<thead>
<tr>
<th>Control (10(^{-4}) A)</th>
<th>5-HT (10(^{-4}) A)</th>
<th>5-HT control (%)</th>
<th>Holding potential (mV)</th>
<th>Clamp pulse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 0.96 0.77 80.2 42</td>
<td>2 2.52 1.03 40.9 34</td>
<td>3 2.40 1.60 66.7 37</td>
<td>4 2.00 1.00 50.0 37</td>
<td>5 0.48 0.18 37.5 45</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Amplitude (mV)</th>
<th>Duration (msec)</th>
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<tbody>
<tr>
<td>30</td>
<td>700</td>
</tr>
<tr>
<td>16</td>
<td>900</td>
</tr>
<tr>
<td>20</td>
<td>500</td>
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Peak slow inward current was measured without making corrections for leakage currents.
After the control $I_{sl}$ had been obtained, $5 \times 10^{-5}$ M of 5-HT was applied. This caused a marked reduction in $I_{sl}$ (Fig. 5-A-b). As seen in Fig. 5-B in which A-a and A-b were superimposed photographically, there was no detectable change not only in the outward current at the end of clamp pulse but also in the tail current observed immediately after the membrane potential was returned from $-12$ mV to $-42$ mV. In all 6 preparations (Table I), the outward current component did not show any detectable change (see also Fig. 8).

In frog atrium, the slow inward current involves slow sodium current ($I_{NaS}$) besides the calcium current ($I_{Ca}$). However, $I_{NaS}$ itself does not contribute to the initiation of contraction. It may be therefore concluded that 5-HT directly reduced $I_{Ca}$.

6. Effect of 5-HT on outward currents

Another possible explanation for the results illustrated in Figs. 1 and 3 is that 5-HT increases the outward potassium current. The outward potassium current consists of time-independent (background) and time-dependent (delayed rectifier) components.

Fig. 6 was an example of results obtained from the experiment (3 preparations) which was designed to investigate the effect of 5-HT on the resting membrane conductance. As illustrated in Fig. 6-A, the effect of 5-HT ($5 \times 10^{-5}$ M) on the action potential was previously confirmed in the same preparation. Record a was taken in Ringer solution and record b was 3 min after an application of 5-HT. Record c was taken 10 min after withdrawal of 5-HT.

Fig. 6. Effect of 5-HT on the resulting membrane conductance. A: Effect of 5-HT ($5 \times 10^{-5}$ M) on the action potential was previously confirmed in the same preparation. Record a was taken in Ringer solution and record b was 3 min after an application of 5-HT. Record c was taken 10 min after withdrawal of 5-HT. B: After the action potential recovered to control, membrane was held at the resting potential level ($-95$ mV) and resting membrane conductance was studied. Record a was taken in Ringer solution and record b was 3 min after an application of 5-HT ($5 \times 10^{-5}$ M). Record c was 10 min after withdrawal of 5-HT.
10^{-5} M) on the action potential was previously confirmed. The membrane potential of this preparation was clamped at the resting potential level (-95 mV) and small rectangular voltage-clamp pulses (less than 5 mV, 500 msec duration) were applied in order to estimate the resting membrane conductance. As demonstrated in Fig. 6-B, 5 \times 10^{-5} M of 5-HT had no detectable effect on the resting conductance. The resting membrane conductance did not show any detectable change even in higher concentrations (1\textendash{}2 \times 10^{-4} M) of 5-HT.

Fig. 7 shows the result obtained from the experiment which was designed to investigate the effect of 5-HT on current-voltage relation near the resting membrane potential. The effect of ACh on the current-voltage relation was also studied in the same preparation in comparison with that of 5-HT. The membrane potential was held at the resting potential level (-95 mV) and rectangular voltage-clamp pulses (500 msec duration) were applied to estimate the membrane slope conductance. In Ringer solution, the current-voltage relation showed anomalous (inward going) rectification demonstrated by closed circles in Fig. 7. After the control current-voltage relation had been obtained, 5 \times 10^{-5} M of 5-HT was applied. 5-HT caused no detectable change in the current-voltage relation, as being demonstrated by open circles; membrane slope conductance near the resting potential is thought to be an indirect indicator of time-independent potassium current.

The current-voltage relation illustrated by square symbols in Fig. 7
Fig. 8. Effect of 5-HT on time-dependent outward current. A: 16 mV depolarizing voltage-clamp pulse was applied from holding potential (-34 mV). Outward tail current was elicited by applying rather long pulse (900 msec). Record 1 was taken in Ringer solution and record 2 was 3 min after an application of 5-HT (5×10^-5 M). B: Outward tail current of A-1 and A-2 was photographically emphasized. Note: There was no detectable change in the degree of activation of time-dependent outward current whereas (I_{si}) was markedly depressed.

Fig. 8 shows the result of the experiment which was designed to investigate the effect of 5-HT on the time-dependent (delayed rectifier) outward current. The preparation (No. 2, Table I) was held at -34 mV and 16 mV depolarization clamp pulses (900 msec duration) were applied to demonstrate the time-dependent outward current. Amplitude of outward tail current was measured to estimate the degree of the activation of time-dependent outward current. After the control outward tail current had been obtained, 5×10^-3 M of 5-HT was applied. 5-HT caused no detectable change in tail current as seen in Fig. 8-B, whereas I_{si} was markedly reduced by 5-HT as seen in Fig. 8-A.

DISCUSSION

The present experiment seems to disclose the mechanism of the initial negative inotropic effect of 5-HT on bullfrog atrial muscles. It demonstrated that the amplitude and duration of the action potential of atrium were de-
pressed by the action of 5-HT. These effects of 5-HT on the action potential appear to be responsible for the initial negative inotropic effect, because these 2 effects were observed simultaneously at the early initial stage of the action of 5-HT. Since depressant effect of 5-HT on both muscle contraction and action potential was not antagonized by atropine in a concentration which completely blocked the comparable depressant effect of ACh, it does not seem to be secondary effect of ACh which might be released from vagal nerve terminals by the effect of 5-HT. Presumably, 5-HT directly acts on muscle cells and depresses their action potentials and thereby inhibits muscle contractions, causing the negative inotropic effect.

Decreases in both the amplitude and duration of the action potential would be caused by an inhibition of the inward Na⁺ or Ca⁺⁺ current and/or an acceleration of the outward K⁺ current. According to the present voltage-clamp analysis, 5-HT does not affect the resting membrane conductance and seems to inhibit selectively the slow inward Ca⁺⁺ current without affecting the inward Na or outward K current during an initiation of the action potential. It may be therefore concluded that 5-HT depresses the slow inward Ca⁺⁺ current of action potentials of atrium and consequently decreases their amplitudes and durations.

In case of mammalian heart, the initial negative inotropic effect of 5-HT was reported to be antagonized by atropine. These results may not be necessarily indicate that 5-HT increases the release of ACh from vagal nerve terminals of these preparations. Indeed, atropine in relatively high concentrations used in these experiments may enhance the slow inward current, and the initial negative inotropic effect of 5-HT was reported not to be antagonized by atropine in the case of rabbit heart.

Since the present experiment was solely concerned to the reduction of Ca⁺⁺ current by the effect of 5-HT, the question whether 5-HT has an indirect sympathomimetic action on frog atrium should be examined by further experiment.

Acknowledgments

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References