DOUBLE-PEAKED POSITIVE CHRONOTROPIC RESPONSES OF ISOLATED AND CROSS-PERFUSED DOG ATRIA TO ATP

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Abstract—The effects of a large amount of adenosine and ATP (100 to 3000 μg) were investigated on sinus rate and developed tension, using the isolated dog atrium which was perfused with arterial blood from a heparinized donor dog. Each of the substances used for study was administered into the cannulated sinus node artery of the isolated atrium. Adenosine caused monophasic negative chronotropic and inotropic effects in a dose-related manner. However, ATP induced two-peaked positive chronotropic phases during a long-lasting negative chronotropic phase, i.e., initially, brief positive (t-1) effects and secondarily, relatively longer positive chronotropic (t-2) effects. These responses were repetitively induced during the experiment. The t-1 and t-2 were not influenced by treatment with propranolol which significantly blocked the positive chronotropic effect of norepinephrine. Aminophylline treatment significantly suppressed t-2 but not t-1. Quinidine (100–1000 μg) did not affect either the t-1 or t-2. It is suggested that ATP-induced tachycardia in the dog is partially due to activation of the P1-purinoceptors named by Burnstock.

Since the report by Drury and Szent-Györgyi (1) in 1929, the cardiac effects of adenyl compounds on pacemaker activity have been reported many times (2–7). Their results demonstrated that adenyl compounds produced a negative chronotropic effect via the P1-purinoceptors named by Burnstock (8). However, there are a few reports of positive chronotropic response to adenyl compounds. Versprille (9) reported that ATP had a positive chronotropic effect on the isolated frog ventricle perfused with normal Ringer's solution. Burnstock and Meghji (10) showed in 1981 that ATP and adenosine induced only a negative chronotropic effect, but ADP and AMP had an initial positive effect followed by a negative chronotropic effect in frog hearts. More recently, Chiba et al. (11) briefly reported that ATP frequently induced double peaked positive chronotropic effects at large doses in the isolated dog atrial preparation which was developed by Chiba et al. (12, 13). In the present study, we used adrenergic beta-blocking agents, propranolol and carteolol, aminophylline and quinidine on ATP-induced effects in order to investigate whether ATP-induced positive chronotropic effects involve stimulation of beta-adrenoceptors, P1-purinoceptors or P2-purinoceptors in the dog atrium.

Materials and Methods
Twenty-eight mongrel dogs of either sex weighing 12–25 kg were anesthetized with sodium pentobarbital, 30 mg/kg, i.v. The right atrium was quickly removed and plunged into Tyrode solution at about 4–10°C. The sinus node artery of the isolated atrium was cannulated with a small polyethylene tubing, put into a blood glass chamber, and then perfused with arterial blood led from the
carotid artery of a heparinized donor dog. The atrial muscle was suspended in a chamber filled with blood at a constant temperature of 37°C and the perfusion pressure was maintained at 100 mm Hg. The sinus rate was recorded with a cardiotachometer (Nihon Kohden RT-5) which was triggered by the action potential waves of atrial electrograms, and the isometric tension developed was measured with a force displacement transducer (Nihon Kohden AP620G). Details of the isolated, blood-perfused canine atrial preparation have been given in previous papers (12, 13). The volume of drug solution injected was 0.01–0.1 ml in a period of 4 sec. Quinidine solution was also continuously administered into the cannulated sinus node artery at a rate of 100 to 1000 µg/min with an infusion-withdrawal pump (Harvard Apparatus 901). Drugs used in this study were adenosine (Kowa Co.), disodium ATP (Kowa Co.), propranolol hydrochloride (Sumitomo Chemicals), carteolol hydrochloride (Ohtsuka Co.), norepinephrine hydrochloride (Sankyo), aminophylline (Eisai), and quinidine sulphate (Tokyo Kasei). Student's t-test was used for comparison of drug data versus control data in all experiments. All values are expressed as means±S.E.

Results
Chronotropic and inotropic effects of large doses of ATP and adenosine: When ATP was administered into the sinus node artery at relatively large doses over 300 µg, two-peaked positive chronotropic phases were frequently observed during a long-lasting negative chronotropic phase. Figure 1 shows a typical tracing of ATP-induced, two-peaked positive chronotropic effects, i.e., an initial brief positive chronotropic phase (t-1) and a secondary positive phase (t-2). The responses to a large dose of ATP were

![Fig. 1. Typical double-peaked chronotropic responses to 300 μg of ATP injected into the sinus node artery of an isolated dog atrium. t-1: Initial tachycardia, t-2: Secondary tachycardia.](image-url)

![Fig. 2. Repetitive chronotropic and inotropic responses to 300 μg of ATP in an isolated atrium of the dog.](image-url)
repetitively induced during the experiment as shown in Fig. 2. Figure 3 shows chronotropic and inotropic responses to increasing doses of ATP and adenosine. A large dose of ATP over 1 mg consistently induced both negative and positive chronotropic effects. The threshold dose for inducing t-1 and t-2 was approximately 300 μg. On the other

Fig. 3. Chronotropic and inotropic responses to increasing doses of ATP and adenosine in an isolated atrium of the dog.

Fig. 4. Chronotropic responses to increasing doses of adenosine and ATP in isolated dog atria. Control sinus rate was 98±8.3 beats/min (mean±S.E.) in 7 preparations.
hand, in all the preparations examined, adenosine produced only negative chronotropic and inotropic effects in a dose range of 100–3000 μg as shown in Fig. 3. Summarized data are shown in Fig. 4. Adenosine caused a maximum decrease in sinus rate at 300 μg, but ATP showed it at 3000 μg.

Effects of propranolol on positive chronotropic responses to norepinephrine and ATP: When norepinephrine was injected into the sinus node artery, positive chronotropic and inotropic effects were dose-relatedly induced from 0.001 μg. Positive chronotropic and inotropic effects of norepinephrine were inhibited by propranolol, but those of ATP were not influenced by it. In 5 atria, effects of 10 μg of propranolol were examined on positive chronotropic and inotropic responses to ATP and norepinephrine as shown in Fig. 5. ATP-induced t-1 and t-2 were not modified by propranolol treatment. Carteolol (10 μg) which has no cardiac depressant action also did not influence ATP-induced t-1 and t-2 in two experiments.

Effects of aminophylline on ATP-induced t-1 and t-2: When aminophylline was injected into the sinus node artery, long-lasting positive chronotropic and inotropic effects were dose-relatedly induced. After treatment with aminophylline, effects of ATP were examined. Figure 6 shows that 1 mg of aminophylline completely inhibited ATP-induced t-2, but t-1 is not completely blocked in this case. In several cases, t-1 was rather slightly enhanced by treatment with aminophylline, although t-2 was usually suppressed. Summarized data are shown in Fig. 7, indicating the blocking effect of aminophylline on the t-2.

Effects of quinidine on ATP-induced t-1 and t-2: When quinidine was injected into the sinus node artery, negative chronotropic and inotropic effects were induced as reported previously (14). A single injection of a large dose of quinidine caused severe depression of sinus rate and the developed
tension. After larger doses of quinidine (1 or 3 mg), ATP frequently induced sinus arrest, and it was very difficult to observe either t-1 or t-2. During a continuous infusion of quinidine (40 μg/min), ATP-induced t-1 and t-2 were not inhibited significantly. Summarized data are shown in Table 1.

Discussion

In 1966, Versprille and van Duyn (15) reported that ATP produced a phase of positive chronotropism that appeared after a negative chronotropic phase in rat hearts. However, they also reported that the positive chronotropic phase was observed after addition of ATP as well as adenosine. In the present experiments, we clearly demonstrated double-peaked positive chronotropic responses to ATP in the dog atrium, but not any positive ones to adenosine. The positive chronotropic responses to ATP and adenosine observed by Versprille and van Duyn (15) might be different from our observed responses because our results were obtained during the negative phase, while their results were obtained after the negative chronotropic responses.

In frog atria, Burnstock and Meghji (10) reported that the responses to adenosine differed from those to ATP. ATP had a biphasic effect, consisting of an initial positive chronotropic phase followed by a negative phase, but adenosine has only a negative chronotropic effect.

Table 1. Effects of quinidine on double-peaked positive chronotropic responses to ATP in isolated dog atria

| Dose of ATP | No. of expts. | A single injection of 1 mg of quinidine<sup>a</sup> | A continuous infusion of 40 μg/min of quinidine<sup>b</sup> |
|-------------|---------------|--------------------------------|--------------------------------------------------|
|             |               | Before | t-1 | t-2 | t-1 | t-2 | Before | t-1 | t-2 | Before | t-1 | t-2 |
| 1 mg        | 6             |        |     |     |     |     |        |     |     |        |     |     |
|             |               | 24±5.8 | 37±11.2 | 18±7.5<sup>c</sup> | 26±9.6<sup>c</sup> |        |     |     |        |     |     |
|             |               |        |     |     |     |     |        |     |     |        |     |     |
| 1 mg        | 3             | 15±6.4 | 33±10.7 | 27±12.8<sup>c</sup> | 47±8.8<sup>c</sup> |        |     |     |        |     |     |

<sup>a</sup>Control sinus rate is 90±10.2 beats/min (mean±S.E.) in 6 dog atria. After quinidine treatment, it is 81±9.0 beats/min. <sup>b</sup>Control sinus rate is 98±10.9 beats/min (mean±S.E.) in 3 dog atria. During quinidine infusion, it is 9.2±11.8 beats/min. <sup>c</sup>No significant difference compared to control values.
tropic effect, similar to our results in dog atria. They also reported that the excitatory action of ATP was unaffected by pretreatment with theophylline, and considered that the excitatory effects of ATP were not mediated via P₁-purinoceptors, but rather by P₂-purinoceptors which are more sensitive to ATP than to adenosine. In the present study, a large amount of ATP induced clear, double-peaked positive chronotropic effects. The secondary positive chronotropic effect (t-2) was significantly suppressed by treatment with aminophylline, but t-1 was not. Therefore, the t-2 response might be mediated via a purinergic P₁-receptor in dog atria, differing from frog atria. On the other hand, in this study, we also used the P₂-purinoceptor blocking agent, quinidine, which was introduced by Burnstock (8). However, the used doses of quinidine never showed selective blocking action on ATP-induced double-peaked positive chronotropic effects, although a large dose of quinidine was not used because of the strong cardiac depressant properties that it has by itself. It is necessary to use a selective P₂-purinoceptor blocking agent which has no cardiac depressant action. Thus, it is not clear yet whether the initial brief positive chronotropic phase (t-1) is mediated via purinergic P₂-receptors or not.

In the past, there have been a large number of papers published on negative chronotropism. As has been reported since the study by Drury and Szent-Gyögyi (1921), the negative chronotropic response to adenylic compounds is readily produced with a small amount of adenosine or adenine derivatives. The negative chronotropic effect might be mediated by purinergic P₁-receptors because it has been reported that the negative chronotropic effect was blocked by P₁-purinoceptor blocking agents, theophylline or caffeine (4, 6, 16–18). In the present study, the negative chronotropic effects induced by ATP were not significantly influenced by aminophylline. Because extremely large doses of ATP were used as an agonist, it is impossible to use such large doses of antagonists for ATP-induced actions.

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