

Contribution of ATP-Mediated Positive Feedback to Sympathetic Nerve-Induced Positive Inotropy in Guinea Pig Ventricular Myocardium

Shogo Hamaguchi,* a,† Marin Kariya, a Aya F. Ozaki, b Iyuki Namekata, a and Hikaru Tanaka a

a Department of Pharmacology, Toho University Faculty of Pharmaceutical Sciences; 2–2–1 Miyama, Funabashi, Chiba 274–8510, Japan; and b College of Pharmacy, Western University of Health Sciences; 309 East Second Street, Pomona, CA 91766, U.S.A.

Received September 11, 2020; accepted December 12, 2020

The functional role of ATP released from sympathetic nerve terminals was examined in isolated guinea pig ventricular papillary muscles. The contractile force of papillary muscles was increased by field electrical stimulation of sympathetic nerve endings. This increase was attenuated by pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) or suramin, blockers of the P2X receptor, and was abolished by propranolol and prazosin. PPADS, suramin, and ATP affected neither the basal contractile force nor the positive inotropic effect of noradrenaline. These results provide functional evidence that ATP released from sympathetic nerve terminals enhances noradrenaline release and contributes to sympathetic nerve-induced inotropy.

Key words ATP; noradrenaline release; sympathetic nerve; guinea-pig; ventricle

INTRODUCTION

The sympathetic nervous system is the major regulator of cardiovascular function. It regulates the contraction of the myocardium and vascular smooth muscle through release of not only noradrenaline but also ATP. Sympathetic purinergic cotransmission has been extensively investigated in blood vessels, and to a lesser extent, in the heart. 1) Released ATP is considered to have both postjunctional effects on the muscle to directly affect contraction and prejunctional effects on the nerve terminals to affect transmitter release. In the myocardium, the effect of ATP appears to vary depending on the animal species and the condition of the heart. 2) Exogenously applied ATP was reported to increase contractile force in the mouse and rat myocardium, 3) while it had no effect on the guinea pig ventricular myocardium. 4) Concerning the prejunctional effect of ATP, it was reported in the guinea pig isolated atria 5) and heart synaptosomes 6) that ATP released from sympathetic nerves enhance the release of noradrenaline through a positive feedback mechanism. RT-PCR studies 5) and Ca 2+ current measurements 7) confirmed the presence of P2X receptors in neurons of the super cervical ganglia. It was postulated that stimulation of neuronal P2X receptors increases intracellular Ca 2+ concentration and enhances noradrenaline release from nerve terminals. Similar observations have also been reported in the rat. 8) However, to what extent such a positive feedback mechanism of ATP contributes to the sympathetic nerve-induced positive inotropy has not been clarified.

In the present study, we intended to pharmacologically clarify whether and to what extent the ATP-mediated positive feedback mechanism contributes to the overall sympathetic nerve-induced positive inotropy. We chose the guinea pig ventricular papillary muscle, which lacks direct inotropic response to ATP, 9) so that the contribution of released ATP to nerve stimulation-induced inotropy could be evaluated with contractile force measurements.

MATERIALS AND METHODS

The present study was conducted in accordance with the “Guiding Principles for the Care and Use of Laboratory Animals” approved by The Japanese Pharmacological Society and the Guide for the Care and Use of Laboratory Animals at Faculty of Pharmaceutical Sciences, Toho University. Hearts were removed from male Hartley strain guinea pigs weighing 300 to 500 g under deep isoflurane anesthesia. Right ventricular papillary muscles were placed in an organ bath filled with a physiological salt solution of the following composition: 118.4 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl 2, 1.2 mM MgSO 4, 1.2 mM KH 2PO 4, 24.9 mM NaHCO 3, and 11.1 mM glucose (pH 7.4, 37°C). For the measurement of contractile force, the preparations were attached to a force-displacement transducer (TB-611T, Nihon Kohden, Tokyo, Japan) connected to a computer amplifier (AP-621G, Nihon Kohden). The output of the amplifiers was digitized and analyzed (Power Lab System, AD Instruments, Dunedin, New Zealand).

The myocardial tissue and the sympathetic nerves were stimulated differentially through platinum plate electrode pairs by an electronic stimulator SEN-330i (Nihon Kohden). The myocardium was stimulated at 1 Hz by 3 ms square pulses of 1.5× threshold voltage. The sympathetic nerves were stimulated by 8 consecutive square pulses of 50 μs duration and 10× threshold voltage applied at 5 ms intervals just after the muscle stimulation.

All experimental data were expressed as the mean ± standard error of the mean (S.E.M.) and statistical significance of differences between means were evaluated by one-way ANOVA followed by the Dunnett’s test for multiple comparisons. A p value less than 0.05 was considered significant.

Pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS; tetrasodium salt) and suramin (sodium salt) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), and noradrenaline (bitartrate salt), 2'-Deoxy-N6-methyl adenosine 3',5'-diphosphate (MRS2179; diammionium

* To whom correspondence should be addressed. e-mail: shogo.hamaguchi@phar.toho-u.ac.jp

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salt), ATP (magnesium salt), propranolol, and prazosin were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Prazosin was dissolved in dimethyl sulfoxide and all the other chemicals were dissolved in distilled water.

RESULTS

Nerve stimulation resulted in an increase in contractile force (Figs. 1Aa, B); the nerve stimulation-induced increase was 177.3 ± 18.9% (n = 24) of the basal value. The nerve stimulation-induced increase in contractile force was abolished in the presence of propranolol and prazosin indicating that the increase was produced by noradrenaline released from sympathetic nerve terminals (Figs. 1Ab, B). The nerve stimulation-induced increase in contractile force in the presence of both 10⁻⁶M propranolol and 10⁻⁶M prazosin was 4.0 ± 1.8% (n = 5) of that in their absence. The basal contractile force was not affected by propranolol and prazosin indicating the absence of neuronal influence under non-nerve stimulating condition; the basal contractile force in the presence of both 10⁻⁶M propranolol and 10⁻⁶M prazosin was 100.9 ± 1.5% (n = 5) of that in their absence.

To assess the contribution of ATP-mediated feedback, the effect of nerve stimulation was performed in the presence of 10⁻⁵M PPADS⁹ and 10⁻⁴M suramin,¹⁰ antagonists of the P₂X receptor (Figs. 1Ac, d, B). The nerve stimulation-induced increase in contractile force was moderately but significantly reduced in the presence of PPADS or suramin; the nerve stimulation-induced increase in contractile force was 79.9 ± 3.9% (n = 7) and 75.4 ± 2.2% (n = 5) of that in the absence of PPADS and suramin, respectively. These results indicate that ATP released from sympathetic nerve terminals stimulates P₂X receptors on the nerve terminals and enhances noradrenaline release. MRS2179 (3 × 10⁻⁵M),¹¹ an antagonist of P₂Y₁ receptor, had no effect on the nerve stimulation-induced increase in contractile force indicating that the P₂Y₁ receptor is not involved in this mechanism (Figs. 1Ae, B).

To confirm that the effects of PPADS, suramin, and ATP described above are not the result of their direct effects on the myocardium, the effects of these agents on the basal contractile force and noradrenaline-induced inotropy were examined. Neither PPADS, nor suramin, nor ATP affected the basal contractile force; the basal contractile force in the presence of 10⁻⁵M PPADS, 10⁻⁴M suramin, and 10⁻⁴M ATP was 102.3 ± 1.5% (n = 5), 104.3 ± 2.7% (n = 5), and 103.0 ± 1.9% (n = 5) of that in their absence, respectively. Noradrenaline, at concentrations of 10⁻⁸ to 10⁻⁴M, increased the contractile force (Fig. 2). Neither PPADS, nor suramin, nor ATP affected the inotropic effect.

![Fig. 1. Effect of Agents on Nerve Stimulation-Induced Increase in Contractile Force](image1)

![Fig. 2. Effect of PPADS and ATP on Noradrenaline-Induced Inotropy](image2)
DISCUSSION

The objective of the present study was to clarify the contribution of the ATP-mediated feedback mechanism in the sympathetic nerve-induced inotropy. Although it was reported that ATP released from sympathetic nerve terminals can stimulate noradrenaline release,\(^5\) whether and to what extent the released ATP can influence noradrenaline-induced inotropy remained to be clarified. The present results that nerve stimulation-induced increase in contractile force was significantly reduced by PPADS or suramin clearly indicated that ATP released from sympathetic nerve terminals could indeed affect sympathetic nerve-induced inotropy through a positive feedback mechanism.

Two other possible explanations for the observed inhibitory effects of PPADS and suramin could be excluded in case of the present study. Firstly, released ATP might have direct positive inotropic effects on the myocardium. In the case of the guinea pig ventricle, we confirmed that ATP has no effect on contractile force, which agrees with an earlier study.\(^4\) Secondly, ATP might somehow increase the myocardial responsiveness to noradrenaline. Our present results that ATP did not affect the inotropic response to noradrenaline (Fig. 2) excluded such possibility. Thus, it was concluded that ATP contributed to the nerve stimulation-induced positive inotropy through enhancement of noradrenaline release. To the best of our knowledge, this is the first report providing evidence that ATP-mediated positive feedback mechanisms affect sympathetic nerve-induced inotropy.

The present results revealed that the ATP-mediated positive feedback mechanism for noradrenaline release accounts for about 20% of the nerve stimulation-induced increase in contractile force. This implies that the feedback mechanism has certain impact on myocardial function. Release of noradrenaline as well as ATP from sympathetic nerve terminals is reported to increase under conditions such as myocardial ischemia,\(^1\) hypertension\(^2\) and diabetes mellitus.\(^3\) Some researchers postulate that pharmacological blockade of the ATP-mediated positive feedback may be beneficial.\(^1\) The pathological role of ATP-mediated feedback mechanism under various conditions and the effectiveness of pharmacological interventions remain to be investigated.

**Acknowledgments** This study was supported in part by JSPS KAKENHI Grant Numbers JP20K16013, JP20K07299, and JP20K07091.

**Conflict of Interest** The authors declare no conflict of interest.

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