# Role of ATP as a Sympathetic Nervous System Transmitter in the Smoothing of Rapid Arterial Pressure Changes

**I. M. Rodionov, A. N. Kosyakov, O. S. Tarasova, and E. N. Timin***

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 11, pp. 461-464, November, 1995
Original article submitted December 12, 1994

Arterial pressure lability and its variations were examined in unrestrained rats following selective elimination of adrenergic or purinergic sympathetic influences on the circulatory system. Both the $\alpha_1$-adrenoceptor blocker prazosin and the nonselective α-adrenoceptor blocker phentolamine lowered the arterial pressure without affecting its lability. When $P_{2x}$ purine receptors were desensitized with α,β-methyleneATP, the resulting pronounced hypotension was accompanied by a two-fold increase in the lability of mean arterial pressure.

**Key Words:** *ATP; awake rats; arterial pressure lability; sympathetic nervous system*

It has been established that, apart from norepinephrine, neurotransmitters for postganglionic sympathetic neurons are adenosine triphosphate (ATP) and certain neuropeptides [1,9,10]. In experiments with isolated vessels, the ATP secreted during sympathetic stimulation was found to cause vasoconstriction and to do so more rapidly than norepinephrine and in phases [10]. However, the significance of the joint action of these two transmitters for the regulation of blood circulation at the organismic level remains unknown. Studies have shown that the carotid sinus pressor reflex is resistant to adrenergic blocking agents, but is largely suppressed when the $P_{2x}$-purine receptors mediating purinergic constrictor influences on blood vessels are desensitized [11]. This suggests that ATP as a sympathetic nervous system transmitter is implicated in the baroreflex regulation of arterial pressure.

The major function of baroreceptors is known to be the mitigation of rapid changes in arterial pressure (AP) occurring in the course of daily activities. Denervation of the carotid sinus and aortic reflexogenic zones in animals results in a greatly increased lability of their AP [2,7]. Such animals show respiration-associated AP fluctuations and abnormal blood flow distribution among the vascular beds [3,5,6]. AP lability is also increased in rats with interrupted sympathetic nerve supply, which points to an important role played by the sympathetic nervous system in stabilizing blood pressure [4].

The phasic constrictor responses elicited by ATP [9,10] led us to speculate that the rapid reflex changes in vascular tone smoothing AP fluctuations are purinergic in nature. The study described here was undertaken to check the validity of this hypothesis.

## MATERIALS AND METHODS

The study was carried out on male white rats aged 4-5 months and weighing 270-370 g. Two days before the tests, they were implanted, under Nembutal anesthesia (50 mg/kg intraperitoneally) with polyethylene catheters inserted into the femoral artery to record AP and into the femoral vein to

Chair of Human and Animal Physiology, Department of Biology, Moscow State University; *Laboratory of Cybernetics, A. V. Vishnevskii Institute of Surgery, Russian Academy of Medical Sciences, Moscow (Presented by I. P. Ashmarin, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0011-1083$12.50 

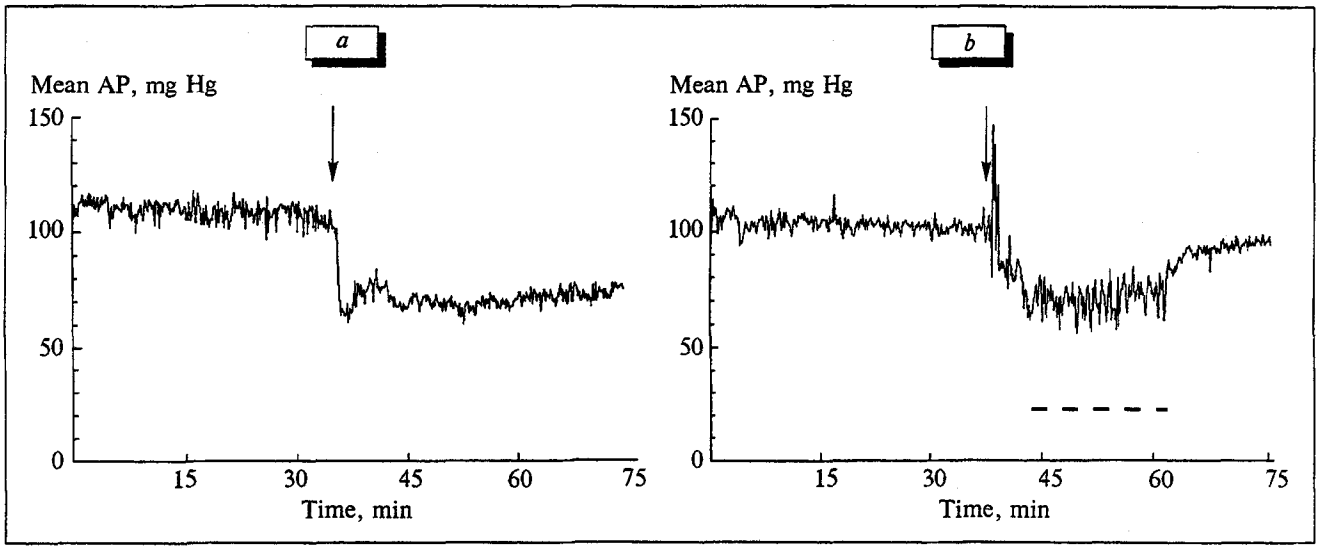


**Fig. 1.** Variations in mean AP (computerized data) in individual rats injected with phentolamine (*a*) and mATP (*b*). Arrows indicate the time of phentolamine injection (*a*) and the time of the first mATP injection (*b*); the period during which purine receptors were completely desensitized is shown by the dashed line; it can be seen that fluctuations in AP during this period were much larger than in the case of similar hypotension caused by phentolamine.

inject the test substances; the catheters were passed under the skin and fixed on the withers. In some rats, the substances were introduced through a catheter passed into the right common carotid artery 4 days before the tests.

On the day of the tests, rats were placed in individual 30×30×30 cm cages; the catheter in the femoral artery was connected to a pressure sensor (Statham P 23AA) and continuously flushed with heparinized (50 units/ml) physiological saline at a rate of 0.2 ml/h to prevent thrombus formation. The catheter for injection was connected to a segment of a polyethylene tube (PE10) 40 cm long so as not to disturb the rat during the injection. All tests were done during evening hours (between 18:00 and 22:00 h) under conditions of limited illumination and auditory stimulation. Data collection was started after a 60-minute adaptation period.

The recorded AP curves were processed beat-to-beat on a computer, at a sampling frequency of 500 Hz. The amplified signal from the sensor was transformed into a digital code (12 bits). The mean value of AP and duration were determined for each cardiac cycle, after which the mean AP and its standard deviation, which served as the measure of AP lability, were calculated for every chosen time interval. From the data thus obtained, frequency distributions of the mean AP values before and during exposure to the test substances were constructed.

Three series of tests were performed. In the first series, rats were injected with the $\alpha_1$-adrenoceptor blocker prazosin (0.1 mg/kg intravenously); control rats received the solvent (0.6% ethanol solution in physiological saline). In the second series, rats were administered the nonselective α-adrenoceptor blocker phentolamine (3 mg/kg intra-arterially); control animals were injected with physiological saline. The injected volume was 0.1 ml/100 g body weight. Data collection was begun 5 min postinjection and continued for 30 min. The efficacy of these two α-adrenoceptor blockers was

**TABLE 1.** Effects of Prazosin, Phentolamine, and mATP on the Mean AP (mm Hg) and AP Lability in Awake Rats ($M \pm \sigma$)

| Substance | No. of rats | Before injection | | After (or during) injection | |
|---|---|---|---|---|---|
| | | AP | AP lability | AP | AP lability |
| Prazosin | 5 | 98.4±2.7 | 4.0±0.5 | 90.2±4.3* | 3.8±0.6 |
| Solvent (control) | 5 | 103.3±4.1 | 4.6±1.0 | 97.9±7.2 | 4.7±1.1 |
| Phentolamin | 4 | 103.6±8.0 | 4.9±1.4 | 74.1±4.4*+ | 4.3±0.7 |
| Saline (control) | 5 | 104.7±7.4 | 4.8±0.9 | 101.7±6.7 | 4.6±0.9 |
| mATP | 5 | 106.6±3.8 | 5.3±1.7 | 81.3±6.1*+ | 8.4±2.1*+ |
| Saline (control) | 5 | 104.7±7.4 | 4.8±0.9 | 102.4±6.8 | 4.8±0.7 |

Note. $p<0.02$: *in comparison with baseline; +in comparison with the control group.

checked in separate tests by injecting rats with phenylephrine intravenously at 2 μg/kg (which raises AP by 35-40 mm Hg). After the prazosin or phentolamine injection, the response to phenylephrine was found to disappear for at least 60 min. In the third series, $P_{2x}$ purine receptors were desensitized with a metabolically stable ATP analog, α,β-methyleneATP (mATP). The latter compound was dissolved in cooled physiological saline and introduced in bolus form into the aortic arch in a volume of 10 μl (0.1 mg) at 1-min intervals, each rat receiving 25 to 30 injections. Control animals each received 10 μl of cooled physiological saline in the same manner.

Before injection of the test substances, AP was recorded in each rat for 30 min to obtain baseline data.

The results were statistically analyzed by the nonparametric Wilcoxon-Mann-Whitney test; the differences were considered to be significant at $p<0.02$.

## RESULTS

The substances used did not cause visible changes in the behavior of rats. In the control groups of rats, no statistically significant changes in AP or AP lability were detected (Table 1).

Prazosin, which selectively blocks adrenergic sympathetic influences on arteries [8], lowered the AP by 8.3±4.0%, while phentolamine produced a greater hypotensive effect (the AP fell by 28.1± ±7.7%), probably by blocking the venous $\alpha_2$-adrenoceptors and thus reducing the venous return [8]. AP lability was not affected by phentolamine (Fig. 1, *a* and Table 1).

After the first injection of mATP, the AP rose by 50-60 mm Hg, after which the pressor responses gradually declined (Fig. 1, *b*). Desensitization of purine receptors developed after the 5th or 6th injection, as could be judged by the disappearance of pressor responses to mATP and the established lower AP level; their desensitization was then sustained by continued mATP injections. It should be noted that when these receptors were desensitized, each subsequent injection of mATP led to a small decrease in AP, and for this reason the 20-sec intervals immediately following its injections were cut out from the records when the data were being processed. The desensitization of purine receptors resulted both in a drop of AP (by 25.5±1.0%) and in its greater lability (Fig. 1, *b* and Table 1); AP fluctuations associated with changes in body posture and higher amplitudes of respiratory waves were observed, as were periodic

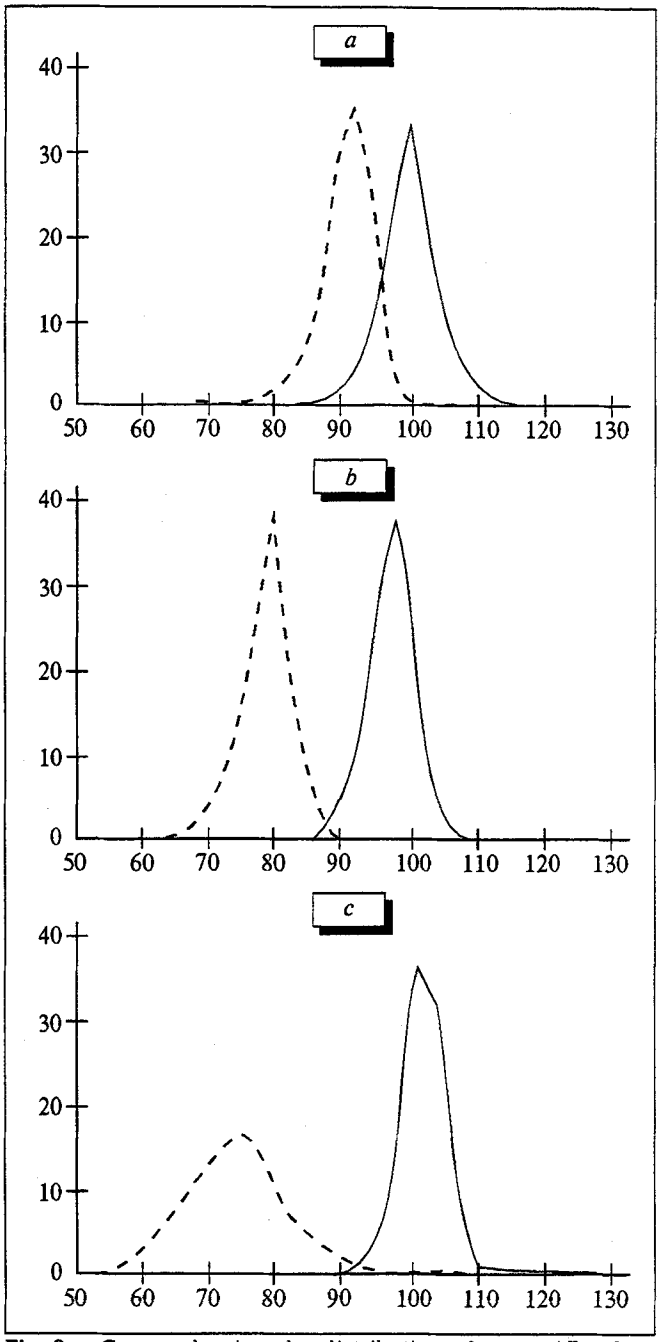


**Fig. 2.** Curves showing the distribution of mean AP values obtained by beat-to-beat analysis in individual rats injected with prazosin (*a*), phentolamine (*b*), and mATP (*c*). Abscissa: mean AP (to construct the distribution curve, the scale was divided in steps of 3 mm Hg); ordinate: number of cardiac cycles in each given interval, as expressed in percent of the total number of cycles during the entire period of recording (30 min). Solid lines show the distributions of AP before (over 30 min) the test substances were injected, while dashed lines show its distributions after (over 30 min) their injection and during the period (20 min) when purine receptors were desensitized.

AP falls, which are characteristic of rats with denervated baroreceptor zones [5,6].

The frequency distributions of mean AP values before and during exposure to prazosin, phento-

lamine, and mATP are shown in Fig. 2. It can be seen that after the injection of the adrenergic blockers the distribution curves merely shifted to the left (because of falls in the mean AP) while remaining bell-shaped (Fig. 2, *a* and *b*). In the case of mATP injections that caused desensitization of purine receptors (Fig. 2, *c*), the distribution curve both shifted to the left and became flatter, reflecting the higher frequency of AP deviations from the mean, i.e., the increased AP labiity.

The lability of the heart rate in rats with denervated baroreceptor zones is reported not to differ from that in control animals [3]. We did not analyze alterations in heart rate lability, but found that its average baseline value for all rats was 376±25 beats/min, that prazosin and phentolamine increased it by 77±26 and 114±24 beats/min, respectively, and that it decreased by 53±42 beats/min after purine receptors were desensitized.

In summary, adrenoceptor blockade and the associated fall in AP did not influence AP labiity, which is consistent with data published earlier [3]. A substantial increase in AP lability occurred only when the purinergic sympathetic influences on vascular smooth muscle were eliminated. The baroreflex regulation of vascular tone, by which rapid AP fluctuations are mitigated, is likely to be mediated by ATP.

This study was supported by grants from the International Science Foundation (MKM 000) and the Russian Foundation for Basic Research (93-04-6489).

## REFERENCES

1. G. Burnstock and C. Kennedy, *Circ. Res.*, **58**, 319 (1985).
2. A. W. Cowley, J. F. Liard, and A. C. Guyton, *Circ. Res.*, **32**, 564 (1973).
3. H. J. Jacob, R. H. Alper, C. L. Grosskreutz, *et al.*, *Amer. J. Physiol.*, **260**, R359 (1991).
4. C. Julien, Z. Q. Zhang, and C. J. Barres, *J. Auton. Nerv. Syst.*, **42**, 1 (1993).
5. B. H. Machado, H. Mauad, and M. L. Glass, *J. Appl. Physiol.*, **72**, 920 (1992).
6. H. Mauad, M. L. Glass, and B. H. Machado, *Hypertension*, **19**, Suppl. 2, II182 (1992).
7. R. A. Norman, T. G. Coleman, and A. C. Dent, *Hypertension*, **3**, 119 (1981).
8. R. R. Ruffolo, *Pharmacol. Biochem. Behav.*, **22**, 827 (1985).
9. N. Sjoblom-Widfeldt, *Acta Physiol. Scand.*, **138**, Suppl. 587, 1 (1990).
10. L. Stjarne, *Rev. Physiol. Biochem. Pharmacol.*, **112**, 1 (1989).
11. O. S. Tarasova and I. M. Rodionov, *Acta Physiol. Scand.*, **146**, 441 (1992).