Role of purinergic cotransmission in the sympathetic control of arterial pressure variability in conscious rats

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Emonnot, Léa, Charles Bakhos, Bruno Chapuis, Valérie Oréa, Christian Barrès, and Claude Julien. Role of purinergic cotransmission in the sympathetic control of arterial pressure variability in conscious rats. Am J Physiol Regul Integr Comp Physiol 291: R736–R741, 2006.—Previous studies have shown that the sympathetically mediated oscillations of arterial pressure (AP), the so-called Mayer waves, are shifted from 0.4 to 0.6 Hz after acute α-adrenoceptor blockade in conscious rats. This raises the possibility that, under physiological conditions, Mayer waves are mediated by the conjoint action of norepinephrine and other sympathetic cotransmitters. To evaluate the possible role of the cotransmitter ATP in determining the frequency of Mayer waves, AP and renal sympathetic nerve activity (RSNA) were simultaneously recorded in 10 conscious rats with cardiac autonomic blockade before and after acute blockade of P2 receptors with pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid. P2 receptor blockade did not alter the mean level and overall variability of AP and RSNA but shifted peak coherence between AP and RSNA from 0.43 ± 0.02 to 0.22 ± 0.01 Hz. A model of the sympathetic limb of the arterial baroreceptor reflex was designed to simulate Mayer waves at 0.2 and 0.6 Hz, with norepinephrine and ATP, respectively, acting as the sole sympathetic cotransmitter. When both cotransmitters acted in concert, a single oscillation was observed at 0.4 Hz when the gain ratio of the adrenergic to the purinergic components was set at 15. The model thus accounted for an important role for ATP in determining Mayer wave frequency, but not in sustaining the mean level of AP or controlling its overall variability.

baroreceptor reflex; Mayer waves; norepinephrine; sympathetic nervous system

There is still some uncertainty about the exact frequency of the sympathetically mediated oscillations of arterial pressure (AP), often termed Mayer waves. In human studies, Mayer wave central frequencies of 0.07–0.12 Hz have been reported (9, 11, 16, 18, 23), and the frequency band containing Mayer waves is typically defined from 0.05 to 0.15 Hz. In rats, Mayer waves have a characteristic frequency of ~0.4 Hz (4, 7, 15). However, when AP power spectra are computed over long (>1-h) periods, Mayer waves do not produce a sharp peak but, rather, appear as a “bump” in the power spectral density function of a given animal and, even more so, when group-average power spectra are calculated (8, 25), which points to substantial intra- and interanimal variability of Mayer wave frequency. This would be inconsequential if AP oscillations in the Mayer band could unequivocally be assigned to a single mechanism. However, because some impingement between cardiovascular control mechanisms in the vicinity of Mayer wave frequency cannot be excluded, questions about the selectivity of the various indexes that can be derived from calculation of spectral power in the Mayer band can potentially be raised (14, 21).

The most commonly accepted mechanism for explaining the generation of Mayer waves is as follows: the sympathetic limb of the arterial baroreceptor reflex tends to be unstable at a particular frequency, called the resonance frequency, and, thus, generates feedback oscillations at this frequency (14). This theory predicts the constancy of Mayer wave frequency within a given species, because the resonance frequency depends on species-specific characteristics of the baroreceptor reflex, such as sympathetic nerve conduction times and frequency responses of resistance vessels to sympathetic modulation. One intriguing possibility, however, is that some of these characteristics might vary over time within a given individual and, even more so, between individuals. In most vascular beds, sympathetic nerve endings release ATP as a cotransmitter of norepinephrine (NE) (20). The kinetics of vasoconstriction evoked by P2X receptor stimulation is faster than the kinetics evoked by α-adrenoceptor stimulation (1), especially because P2X receptors are ligand-gated ion channels (27), whereas α-adrenoceptors are G protein-coupled receptors. Accordingly, acute α-adrenoceptor blockade shifts Mayer waves and accompanying oscillations of renal sympathetic nerve activity (RSNA) from 0.4 to ~0.6 Hz in conscious rats (3). Reciprocally, P2 receptor blockade eliminates 0.4-Hz AP oscillations, whereas it enhances AP oscillations at lower frequencies, especially in the 0.15- to 0.2-Hz range (10). In the latter study, however, the sympathetic nature of these slower oscillations was not established, inasmuch as sympathetic nerve activity was not recorded.

The primary objective of the present study was to determine whether P2 receptor blockade actually induces the appearance of slow sympathetically mediated oscillations of AP. For this purpose, RSNA and AP were simultaneously recorded in conscious rats before and after acute P2 receptor blockade. The second aim of the study was to describe a model of the sympathetic limb of the arterial baroreceptor reflex involving the corelease of two sympathetic transmitters acting with different time constants and, thus, potentially producing Mayer waves at two distinct frequencies. Then we examined whether the model would simulate the genesis of a single oscillation at an intermediate frequency when both transmitters were operating in concert.

METHODS

Animals and surgery. Ten male Sprague-Dawley rats (Charles River Laboratories, L’Arbresle, France; 310–330 g body wt, 9–10 wk

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of age) were housed individually with free access to food and water and maintained on a 12:12-h light-dark cycle. On completion of the experiments, the rats were euthanized with an intravenous overdose of pentobarbital sodium. All experiments were performed in accordance with the guidelines of the French Ministry of Agriculture for animal experimentation and were approved by the local Animal Ethics Committee.

As previously described in detail (6), the rats were anesthetized with 1.5–2% halothane in oxygen, and femoral arterial and venous polyethylene catheters were inserted for AP measurement and drug administration, respectively. Then, under pentobarbital sodium (60 mg/kg ip) anesthesia, a major branch of the left renal nerve was placed on a bipolar electrode and insulated with silicone gel for RSNA measurement. Both catheters and the electrode cable were led subcutaneously to exit at the back of the neck. The rats received a single injection of penicillin G (50,000 IU ip) and were allowed 14–16 h to recover from anesthesia.

**Experimental protocol in conscious rats.** All experiments were performed while the rats were under cardiac autonomic blockade (atropine methyl nitrate and atenolol, 2 mg·kg⁻¹·h⁻¹ iv each), which prevented the potential interference of heart rate fluctuations in the genesis of Mayer waves (2, 5, 24). AP and RSNA were recorded before and after acute blockade of P2 receptors with pyridoxidal phosphate-6-azophenyl-2,4'-disulfonic acid (PPADS) (27). PPADS was administered intravenously in a total dose of 37 mg/kg (12 mg/kg as a bolus injection, followed by a 60-min continuous infusion of 25 mg·kg⁻¹·h⁻¹). This dose was chosen on the basis of preliminary experiments performed on three urethane-anesthetized, artificially ventilated rats (5, 24) with bilateral cervical vagotomy. Vagotomy was performed because stimulation of P2X receptors on vagal afferent nerves evokes a Bezold-Jarisch reflex comprising hypotension and bradycardia (22), which blurs the direct vasococontractor effect. The pressor response to the P2X receptor agonist α,β-methylene ATP (25 μg/kg iv) was measured at regular intervals before and after initiation of PPADS administration (see RESULTS).

**Data collection and analysis.** For measurement of AP, the arterial catheter was connected to a precalibrated pressure transducer (model TWF-R, Ohmeda, Bilthoven, The Netherlands) coupled to an amplifier (model 13-4615-52, Gould). RSNA was amplified (×50,000), bandpass filtered (300–3,000 Hz; model P-511J, Grass Instruments, Quincy, MA), and rectified (analog homemade rectifier, including a low-pass filter with a cut-off frequency of 150 Hz). A computer equipped with an analog-to-digital converter (model AT-MIO-16, National Instruments, Austin, TX) and LabVIEW 5.1 software (National Instruments) were used to sample the AP and RSNA signals at 500 and 5000 Hz, respectively.

Under baseline and P2 blockade conditions, 12-min periods were selected for further analysis. The background noise (postmortem activity) was subtracted from the RSNA signal, which was then resampled at 500 Hz by calculation of average values over 10 consecutive points. All values were normalized by the mean RSNA value calculated over the baseline period. From each 721-s period, 21 data sets of 32,768 points (65.54 s) overlapping by half were processed. For each data set, power spectral density was computed using a fast Fourier transform algorithm after subtraction of the mean, removal of the linear trend when significant, and application of a Hanning window. The spectra obtained for the 21 data sets were then averaged. The frequency resolution was 0.01526 Hz. Spectral powers were calculated by integration in the low-frequency (0.015 to 0.259 Hz), midfrequency (MF, 0.275 to 0.748 Hz), and high-frequency (0.763 to 2.5 Hz) bands.

Cross-spectral techniques were used to compute the coherence function between RSNA and AP. Coherence values >0.206 indicate a statistically reliable \( P < 0.05 \) linear relation between oscillations of the two signals (2).

**Numerical simulations.** The closed-loop model of the sympathetic limb of the arterial baroreceptor reflex was implemented using the Matlab/Simulink software package (The MathWorks, Natick, MA).

**Statistics.** Values are means ± SE. Paired comparisons (P2 receptor blockade vs. baseline) were made by Wilcoxon’s signed rank test. \( P < 0.05 \) was taken to indicate statistical significance.

**RESULTS**

**Effectiveness of P2 receptor blockade.** During PPADS administration, the pressor effect of α,β-methylene ATP was progressively blunted, and the maximum reduction was observed 75 min after initiation of the infusion (Fig. 1). At this time, the pressor effect was 12 ± 1% of the control response.

**Effect of P2 receptor blockade on AP, RSNA, and their variabilities.** According to the kinetics of the inhibitory action of PPADS, the period used to analyze the effect of P2 receptor blockade was initiated 57 ± 3 min after initiation of the PPADS treatment. In the 10 rats of the study, baseline values of mean AP, heart rate, and RSNA (114 ± 3 mmHg, 366 ± 6 beats/min, and 1.53 ± 0.20 μV) were not significantly altered (111 ± 4 mmHg, 356 ± 6 beats/min, and 1.67 ± 0.22 μV) by PPADS administration. The overall variabilities (total powers) of AP and RSNA also remained unchanged (Table 1). The only significant effect of P2 receptor blockade on AP variability was an ~70% reduction in the MF power (Table 1), which resulted from the disappearance of the peak centered at ~0.4 Hz (Fig. 2A). In terms of RSNA variability, low-frequency power was increased and MF power was decreased after P2 receptor blockade (Table 1), mainly because of a shift of a major oscillation from ~0.4 to ~0.2 Hz (Figs. 2B and 3).

Under baseline conditions, coherence between AP and RSNA reached a maximum (0.89 ± 0.02) at 0.43 ± 0.02 Hz. After P2 receptor blockade, peak coherence (0.86 ± 0.02) was observed at 0.22 ± 0.01 Hz (Fig. 2C).

**Simulation of AP Mayer waves.** A model of sympathetic cotransmission (Fig. 4) was designed to simulate Mayer waves.
at various frequencies depending on whether NE and ATP acted alone or in concert. According to previous findings (4, 8, 15, 29), the adrenergic and purinergic components of vascular sympathetic transmission were modeled as low-pass filters combined with fixed time delays. Numerical values for the time delays were taken from the in vitro study of Bao et al. (1), which reported the latency of the contractile response of the rat tail artery to short trains of impulses in the presence of selective blockers of either transmitter (0.16 and 0.58 s for purinergic and adrenergic components, respectively). The model incorporated the transfer function of central baroreflex pathways that had been identified in a previous study (24) (see Appendix). The characteristics of each filter were then determined by running simulations so that the baroreflex loop became unstable, i.e., generated self-sustained feedback oscillations, at 0.2 Hz (adrenergic component) or 0.6 Hz (purinergic component; Fig. 5, A and B). Finally, simulations were run such that both cotransmitters were involved in vascular neurotransmission. Although the relative importance of NE and ATP was varied, a single feedback oscillation was observed. Its frequency was 0.4 Hz when the ratio of static gain of the adrenergic component to static gain of the purinergic component was set at 15, i.e., when the functional importance of the adrenergic component was 15 times greater than that of the purinergic component under static conditions (Figs. 5C and 6).

**DISCUSSION**

The results of the present study support the conclusion that the relative contribution of NE and ATP to vascular sympathetic neurotransmission is a major determinant of Mayer wave frequency. Furthermore, the modeling analysis suggests that, under physiological conditions in rats, this relative contribution largely favors NE, a finding that is compatible with the obser-

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**Table 1. Effect of P2 receptor blockade on spectral powers of AP and RSNA in conscious rats**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>P2 blockade</th>
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<tr>
<td><strong>AP, mmHg</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total power</td>
<td>13.8±3.9</td>
<td>12.9±2.0</td>
<td>0.647</td>
</tr>
<tr>
<td>LF power</td>
<td>8.2±2.9</td>
<td>10.8±1.9</td>
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<tr>
<td>HF power</td>
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<tr>
<td><strong>RSNA, NU</strong></td>
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<td></td>
<td></td>
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<tr>
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<tr>
<td>HF power</td>
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<td>4,647±948</td>
<td>0.959</td>
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</table>

Values are means ± SE (n = 10 rats). AP, arterial pressure; RSNA, renal sympathetic nerve activity; LF, low frequency (0.015–0.259 Hz); MF, mid-frequency (0.275–0.748 Hz); HF, high frequency (0.763–2.5 Hz); NU, normalized units. P values refer to comparisons between baseline and P2 blockade conditions.

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**Fig. 2.** Spectral analysis of AP and renal sympathetic nerve activity (RSNA) variabilities in conscious rats. Power spectra of AP (A), RSNA (B), and coherence (C) were computed from 12-min recordings (segmented into 65-s periods) obtained before (thin lines) and after (thick lines) acute P2 receptor blockade with PPADS. Individual RSNA data sets were normalized (normalized units [NU]) by the mean RSNA value calculated over the baseline period. Group-average (n = 10 rats) spectra are shown, and SE lines have been omitted for legibility. Vertical dashed lines delimit midfrequency (0.275- to 0.748-Hz) band.
vation that acute blockade of P2 receptors has little or no effect on the level of AP and its overall variability.

Effect of P2 receptor blockade on mean level and overall variability of AP. Infusion of PPADS resulted in an 80–90% reduction of the pressor response to α,β-methylene ATP in anesthetized vagotomized rats. This peak effect was observed near the end of the drug infusion. Accordingly, AP data from conscious rats that were used for further analysis were taken from a 12-min period over which it was expected that maximum P2 receptor blockade had been achieved. At that time, PPADS did not induce a significant change in the mean level of AP, which is consistent with results of a previous study performed on conscious rats (10). Importantly, the RSNA level was also not altered by PPADS administration, demonstrating that the lack of depressor effect of the drug did not result from a reflex sympathetic activation. These results indicate that, at least in quiet conscious rats, the release of ATP by sympathetic nerves plays a minor role in sustaining systemic vascular resistance and AP. On the contrary, release of NE acting at vascular α-adrenoceptors has been shown to play a major role in sustaining AP in conscious rats, as demonstrated by the large depressor effect of acute phentolamine administration (3).

Another important observation of the present study is that PPADS administration did not affect the overall variability of AP, as could be estimated by the total power of AP spectra. Total power of AP refers to AP fluctuations within 1-min periods. Over such a time scale, AP fluctuations were not enhanced by P2 receptor blockade, which does not preclude an effect on slower AP fluctuations. Golubinskaya et al. (10) reported that PPADS induced a slight but significant increase in the standard deviation of beat-to-beat mean AP recorded over 30-min periods in conscious rats. In the present study, the standard deviation of AP data resampled at 1 Hz was calculated. This global index of AP variability was not significantly altered by P2 receptor blockade (4.1 ± 0.4 and 4.4 ± 0.3 mmHg before and during PPADS infusion, respectively).

Effect of P2 receptor blockade on Mayer wave frequency. The central frequency of Mayer waves was defined as the frequency at which coherence between AP and RSNA was maximal (14). This strategy was especially well adapted to the present experiment, where P2 receptor blockade lowered this frequency. As a consequence, the “new” AP Mayer waves impinging on preexisting slow oscillations, especially a major AP oscillation centered at 0.1–0.15 Hz. The latter oscillation has been described in several previous studies performed on conscious rats (4, 12, 25) and has been shown to derive mainly from an oscillation of mesenteric vascular resistance (12, 19). Slow oscillations of mesenteric resistance are especially well defined in ganglion-blocked rats, in which AP is maintained by an intravenous infusion of NE but are absent in chronically sympathectomized rats (19). The latter findings indicate that these oscillations are not directly generated by the sympathetic nervous system but, rather, require a noradrenergic vasoconstrictor tone. Golubinskaya et al. (10) also reported that PPADS amplified AP power in the 0.15- to 0.2-Hz frequency range.
range and that this effect was abolished by phentolamine. They interpreted this finding as a shift of sympathetically mediated oscillations of AP toward lower frequencies, but they did not consider the possibility that suppression of \(\alpha_1\)-adrenoceptor vasoconstrictor tone might have dampened a slow preexisting oscillation. In the present study, we directly demonstrate that P2 receptor blockade effectively lowers the frequency of coherently oscillations of RSNA and AP, although the effect on the AP oscillation is hardly visible because of the presence of slow preexisting oscillations.

**Modeling sympathetic cotransmission.** The model assumed costorage and corelease of NE and ATP in most vascular beds, which is supported by current experimental evidence (26). The dynamic characteristics of the contractile response evoked by each transmitter alone could not be entirely inferred from the existing data in the literature. Numerical values for the fixed time delays of the transfer functions were those reported by Bao et al. (1) for the rat tail artery. The characteristics of the low-pass filter used in the model were those allowing simulation of feedback oscillations at the frequency of spontaneous Mayer waves, i.e., 0.2 Hz for the adrenergic component and 0.6 Hz for the purinergic component of sympathetic transmission. The natural frequency of the filter of the adrenergic transfer function was markedly lower than that of the purinergic transfer function (0.04 vs. 0.25 Hz), which is consistent with the differences reported by Bao et al. (1) for the kinetics of the contractile response of the rat tail artery when it is mediated by P2 receptor, \(\alpha_1\)-adrenoceptor, or \(\alpha_2\)-adrenoceptor stimulation (time to peak contraction = 0.95, 3.1, and 7.9 s, respectively).

Then we examined whether corelease of NE and ATP would result in two feedback oscillations at 0.2 and 0.6 Hz or in a single oscillation at an intermediate frequency. We found that whatever the relative importance of the adrenergic and purinergic components (modeled as the ratio of static gain of the adrenergic transfer function to static gain of the purinergic transfer function), the coexistence of two distinct oscillations at or near 0.2 and 0.6 Hz never occurred. On the contrary, a single, well-defined oscillation could be simulated at each value of the gain ratio. Its frequency was 0.4 Hz when this ratio reached 15.

**Conclusions and perspectives.** The model predicts that the functional importance of ATP is \(\sim 15\) times less than that of NE, which is consistent with the lack of effect of acute blockade of P2 receptors on AP and overall variability of AP. The model also predicts that the central frequency of Mayer waves is highly sensitive to changes in the relative contribution of NE and ATP to sympathetic transmission. It is therefore possible that between-species differences in Mayer wave frequency depend, in part, on the role of ATP in vascular sympathetic neurotransmission. Although there have been a few reports about between-species differences in the density of P2X receptors in some vascular beds (20, 27), it is not known whether the functional consequences of these differences are relevant to AP control. The present study also suggests that within- and between-subject variability in Mayer wave frequency might be attributable to changes or differences in the relative importance of NE and ATP in vascular control. In the present study, the spontaneous variability of Mayer wave frequency could not be properly studied, mainly because of the
method used for determining Mayer wave frequency, which was based on coherence analysis. Coherence must be estimated over at least three adjacent periods; thus, because of the short duration of the recordings used for analysis, the maximum number of coherence values that could be obtained was too small. This duration was dictated by the kinetics of the P2 receptor blockade being achieved with PPADS. With use of other methods for blocking P2 receptors, future studies will have to examine whether P2 receptor blockade effectively reduces the spontaneous variability of Mayer wave frequency.

**APPENDIX**

According to a previous study (24), the transfer function of central baroreflex pathways ($H_c$) was described by the following equation

$$H_c(\omega_f) = \frac{K}{1 + 2j\lambda(\omega_f - \omega_0) + jf_0^2} \exp(-2\pi Tf_f)$$

where $j$ and $f$ are the imaginary operator and frequency (Hz), respectively, $K$ is a static gain (arbitrarily set to 1), $f_0$ is the corner frequency of a derivative gain (0.157 Hz), $f_0$ is the natural frequency (1.12 Hz), $\lambda$ is the damping coefficient (1.71) of a second-order low-pass filter, and $T$ is a fixed time delay (0.101 s).

The transfer functions of the adrenergic and purinergic components of sympathetic vascular transmission ($H_{SATP}$) and $H_{NL}$, respectively were described by the following equation

$$H_I(\omega_f) = \frac{K}{1 + 2j\lambda(\omega_f - \omega_0) + jf_0^2} \exp(-2\pi Tf_f)$$

Numerical values for the fixed time delay were 0.16 and 0.58 s for the purinergic and adrenergic components, respectively (1). To estimate $f_0$ of the low-pass filters, simulations were run (Figs. 4 and 5) so that the baroreflex loop became unstable at 0.2 Hz in the case of the adrenergic component ($f_0 = 0.044$ Hz) or 0.6 Hz in the case of the purinergic component ($f_0 = 0.246$ Hz). For the adrenergic and purinergic transfer functions, $\lambda$ was arbitrarily set to 1.2 (8). $K$ of each transfer function was set exactly at the value leading to the production of self-sustained oscillations. At lower $K$ values, feedback oscillations vanish rapidly; at higher $K$ values, oscillations grow in amplitude indefinitely, unless a nonlinear element is incorporated into the model (17, 28).

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