ATP Modification of Serotonin-Induced Contraction of the Rat Pulmonary Artery (44091)

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Abstract. **Serotonin (5HT) and ATP are simultaneously released from activated platelets at the site of vascular injury and are hypothesized to play a significant role in hemostasis. Our laboratory investigated the modulation of vascular contraction of arterial ring segments by 5HT plus ATP as a model of the potential regulation of localized vascular tone by platelet releasates in regions of arterial damage. This study expands our focus on how these two vasoactive components, released from platelet dense granules, regulate vascular tone. 5HT– and 5HT analog–induced vasoconstrictions were measured in the presence or absence of ATP and ATP analogs with intact or deendothelialized rat pulmonary arterial ring segments suspended in organ baths. The possible prensence of $5HT_2$ and $5HT_{1A}$ receptor types in the rat pulmonary artery was demonstrated by vasoconstrictions induced by 5HT and (+)-8-hydroxy-2-(di-N-propylamino) tetralin hydrobromide (DPAT). The DPAT response was only 30%–50% of that induced by comparable concentrations of 5HT. The 5HT-induced contraction was inhibited by the $5HT_2$ antagonist, ketanserin. ATP equally relaxed 5HT and DPAT contracted tissue while the P_{2X} agonist, αβ-methylene ATP, increased the contracted state of DPAT-treated arteries to a significantly greater extent than observed with 5HT. The P_{2Y} agonist, 3′-*O*-(4-benzoyl)benzoyl ATP (BzATP), the P_{2X} agonist βγ-methylene ATP, and ATP all relaxed 5HT-induced contractions to similar levels under a number of physiological conditions. The final level of 5HT-induced tissue contraction was the same whether ATP was added prior to, after, or simultaneously with 5HT. ATP and the phosphodiesterase inhibitor, theophylline, inhibited 5HT-induced vasoconstriction in an additive fashion. The ATP effects were endothelium dependent, while the inhibition by theophylline was not. The distribution of 5HT and ATP receptor types, as indicated by these and numerous other studies, appears to vary within different regions of the cardiovascular system. Extracellular ATP can synergistically enhance or inhibit 5HT-contracted blood vessels differentially at localized regions, which would significantly impact on localized vascular tone, and this in turn may modulate hemostasis and thrombosis.** [P.S.E.B.M. 1997, Vol 214]

Blood flow rates, shear forces, blood vessel geometry, and platelet reactivity contribute to normal hemostasis (1). Among the host of potential endogenous vasomodulators, activated platelets secrete several that can alter regional blood vessel geometry and participate in hemostasis. These platelet factors include thromboxane, serotonin, and ATP. The question addressed in this study is what cumulative effect might the latter two vasomodulators have on localized regions of blood vessels where ATP concentrations can exceed 20 μ*M* (2).

Serotonin (3) induces a receptor-mediated vasoconstriction. However, depending upon receptor type occupancy (4) and the physical state of the blood vessel (5, 6), serotonin may also induce vasodilation. Serotonin appears to play a significant role in hemostasis (7–9), hypertension (3, 10), and vascular tone (5, 6, 10, 11). A number of different 5HT receptor types and subtypes

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Received July 2, 1996. [P.S.E.B.M. 1997, Vol 214]
Accepted September 27, 1996.

0037-9727/97/2143-0233$10.50/0

have been described in a variety of cells (12–14). The current 5HT receptor designations include $5HT_1$, $5HT_2$, $5HT_3$, $5HT_4$, and subtypes $5HT_{1A}$, $5HT_{1B}$, $5HT_{1C}$, and $5HT_{1D}$. Some of the receptor types and functions have been resolved pharmacologically with selective agonists and antagonists (12–15), as well as by cloning the receptor genes (16).

Extracellular ATP inhibits agonist-induced platelet aggregation (17, 18) and intracellular calcium mobilization (19) *via* a purinoceptor–G protein coupled pathway. Extracellular ATP has also been shown to regulate vascular tone (11, 20) in addition to numerous other physiological effects (2). ATP can function as a cellular modulator, a neurotransmitter (21), or a co-transmitter (22). Many of the contractile effects of ATP on the cardiovascular system may be the summation of ATP acting as a neurotransmitter plus the direct stimulation of receptors on smooth muscle cells, or counterbalanced by the ATP-induced release of the vasodilating endothelium-derived relaxing factor (23). Several major sources of extracellular ATP, and its metabolites, in the cardiovascular system have been identified. These include ATP released from (i) endothelial cells during hypoxia (20, 23) and injury (24); (ii) activated platelets (24); (iii) damaged red blood cells (25); (iv) red blood cells and cardiac muscle during hypoxia (26); and (v) nerve terminals (27). Extracellular ATP elicits biological responses through specific P_2 purinoceptors. ATP and its nonhydrolyzable structural analogs have been employed to characterize P_2 purinoceptors. Two major subtypes were initially identified, P_{2x} and P_{2y} (20, 23), with several others being subsequently described (28). A rank order of agonist sensitivity was established for the P_{2x} receptor of αβ-methylene ATP (αβATP), βγ-methylene ATP (βγATP) > ATP = 2 me-S-ATP and for the P_{2y} receptor of ATP > αβATP, βγATP (23).

We have previously demonstrated an inhibitory effect of ATP on agonist-induced platelet aggregation (17, 18) and a modulating effect on serotonin-induced arterial contractions (11). These effects of ATP may significantly impact on vascular tone and hemostasis. Studies presented in this report further explore the cumulative interactions of extracellular ATP and 5HT on rat pulmonary arterial contractions *in vitro*.

Materials and Methods

Tissue Preparation. For experiments depicted in Figures 1 and 2, and Table I, tissue was isolated from male Wistar rats that had been sacrificed by decapitation. The remaining data were generated with male Wistar and Long Evans Hooded rats anesthetized with pentobarbital sodium. Variability of data was found to be independent of strain. All rats were approximately 300 g and were being employed by other researchers in the institution. The lung-heart complex was excised immediately after death and placed in an aerated (95% O_2/5% CO_2) $NaHCO_3$/PSS buffer (121.9 m*M* NaCl, 4.7 m*M* KCl, 1.2 m*M* $MgCl_2$, 2.5 m*M* $CaCl_2$, 1.2 m*M* KH_2PO_4, 11.5 m*M* glucose, 24.9 m*M* $NaHCO_3$), pH 7.4. The main pulmonary artery was removed and surgically cleaned of surrounding loose connective tissue, and 2-mm arterial ring segments were employed for the studies.

Arterial Contractions. The 2-mm arterial ring segment was suspended with two stainless steel pins through its lumen, one fixed and the second attached to a Grass force transducer in a fashion similar to that

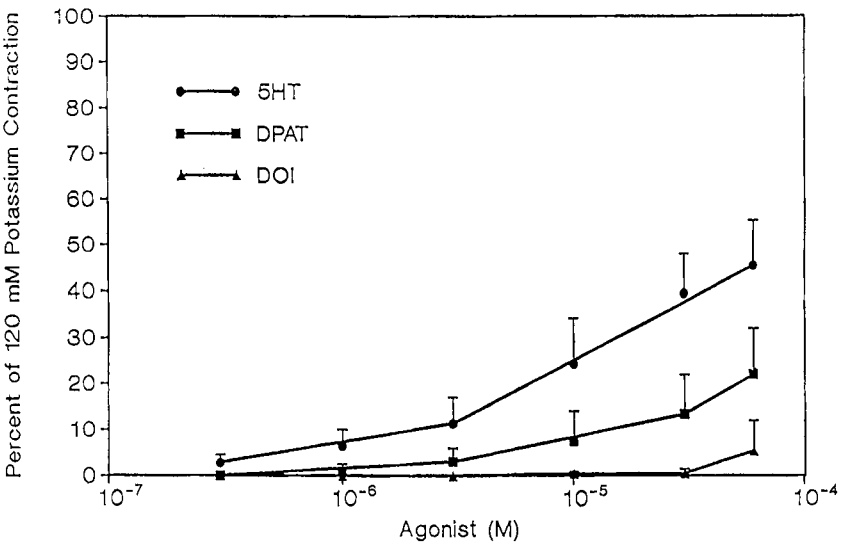

Figure 1. *In vitro* contraction of rat main pulmonary artery induced by increasing concentrations of serotonin (5HT) and its analogs. Arterial rings were hung in tissue baths with a preload tension of 2 g. Tissue contraction is presented as a percentage of KCl-induced contraction. Each experimental point for 5HT and DPAT represent 7–10 determinations with tissue from different animals, and for DOI 4 determinations. Each point represents the mean ± SD.

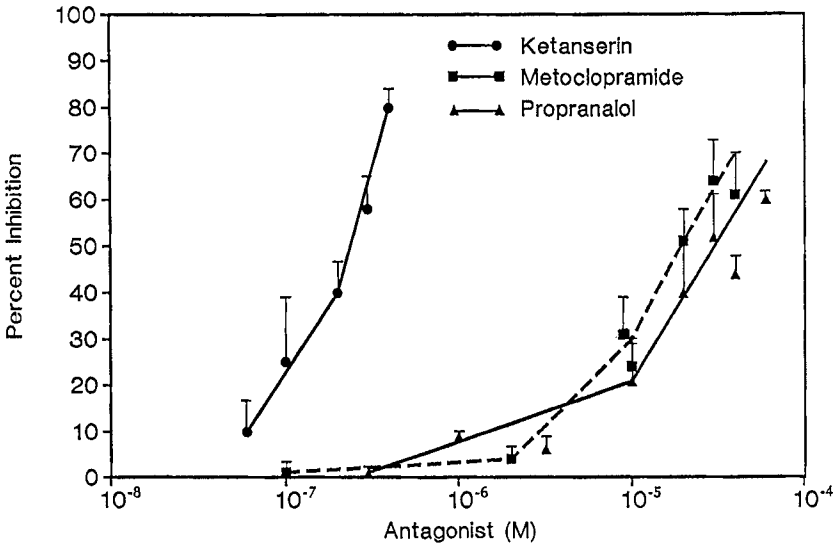

Figure 2. Inhibition of 30 μ*M* serotonin–induced rat pulmonary arterial contraction by serotonin antagonists. Each experimental point in the IC_{50} region represents 6–10 determinations, each with tissue derived from different animals, and all other points 2–4 determinations. Each point represents the mean ± SD.

previously reported (29). The tissue was suspended in a 37°C water-jacketed organ bath containing 20 ml of the $NaHCO_3$/PSS buffer, pH 7.4, with a continuous stream of 95% O_2/5% CO_2. The tissue was slowly stretched, over a period of 1 hr, to a pre-load tension of 2 g, with repeated washings after each 0.5-g increment. This pre-load tension was experimentally found to consistently yield good agonist-induced contractions. After reaching this new steady state, agonists, antagonists, ATP, and ATP analogs were added to a final concentration as indicated in the text. Measurements of responses were terminated by aspiration of the reaction solution from a valve at the bottom of the bath followed by repeated washes until the organ returned to its baseline tension. Measurements were recorded on a Gould chart recorder. In some experiments, the endothelium was removed by rubbing a round toothpick through the lumen of the tissue several times. Response to acetylcholine (Ach) was used to gauge the integrity of the endothelium (relaxation) or deendothelialized (contraction) state of the tissue.

Table I. The Effect of Extracellular ATP on Relaxing and αβATP on Further Contracting 5HT- and DPAT-Induced Rat Pulmonary Arterial Contractions

	% changes of 5HT- and DPAT-induced initial contractions	
	5HT	DPAT
ATP-induced relaxation		
0.18 μ*M* ATP	9.7 ± 2.4 (10)	8.3 ± 4.4 (8)
1.8 μ*M* ATP	42.3 ± 4.3 (12)	38.0 ± 6.3 (9)
g tension	0.57 ± 0.05 (10)	0.40 ± 0.06 (9)
αβATP-induced contraction		
0.2 μ*M*	9.6 ± 2.6 (9)	24.3 ± 3.8 (5)[a]
2 μ*M* αβATP	44.3 ± 9.2 (10)	86.2 ± 25.5 (5)[b]
g tesnion	0.45 ± 0.06 (9)	0.38 ± 0.04 (6)

Note. Data are presented as mean ± SEM (number of determinations).
[a] *P* value relative to 5HT < 0.05 by Student's *t* test.
[b] *P* value relative to 5HT < 0.5 by a paired Student's *t* test with four paired groups, as stated in text.

Serotonin Antagonist Studies. Serotonin antagonist studies were conducted by first contracting the tissue with the agonist (30 μ*M* 5HT) followed by the addition of increasing amounts of inhibitor to the fully contracted tissue. The reverse order of addition of antagonist then agonist to the tissue could not be employed since repeated contractions with 5HT yielded small, but significantly reduced, maximal contractions with each repetition. Our methodology to deal with the 5HT-induced tachyphylaxis was similar to that employed by Cocks and Angus (30), and is likely to yield valid IC_{50}'s for at least ketanserin and propranolol. Propranolol (31) and ketanserin (32) are competitive inhibitors, with propranolol being a fast onset, offset antagonist (31). Furthermore, (−) propranolol was shown to effectively displace agonists from $5HT_1$ and $5HT_{1B}$ receptors while (+) propranolol was essentially inactive (33). We employed (±) propranolol. All of the agonists and antagonists were obtained from Research Biochemicals International Inc. (Natick, MA).

Statistics. All of the data were analyzed by analysis of variance (ANOVA) and the Bonferoni *t* test or by an unpaired Student's *t* test (two sided). Due to large variations encountered in two experiments the agonist-ATP interactions with samples derived from different tissue specimens necessitated, as noted, the analysis of data by a paired Student's *t* test. Experimental determinations—numbers in parentheses in the tables or error

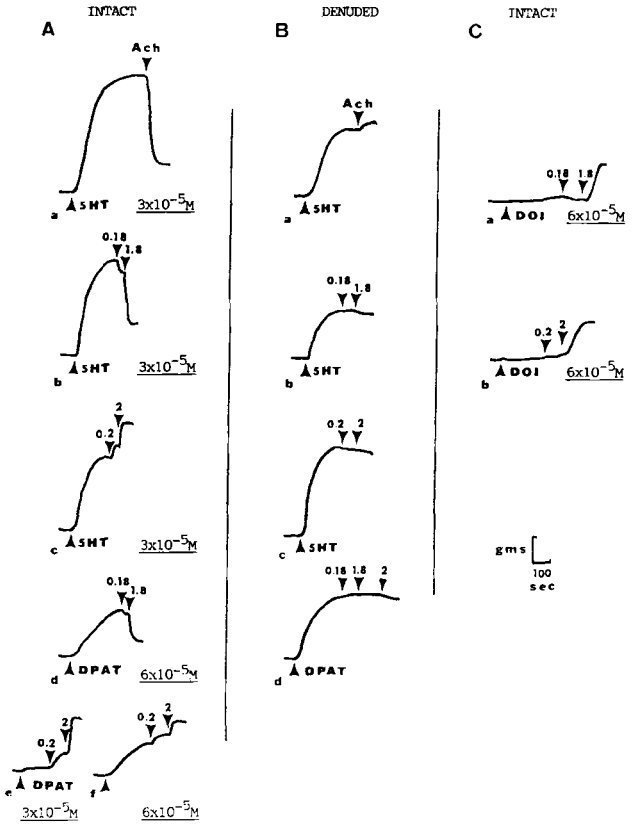

Figure 3. Representative tracings of responses of rat pulmonary arterial ring segments to serotonin (5HT), ATP, and their analogs. (A and C) With intact endothelium. (B) With de-endothelialized tissue. $A_{a,b,c}$ and $B_{a,b,c}$ contracted with 3×10^{-5} *M* 5HT. $A_{d,f}$ and B_d contracted with 6×10^{-5} *M* DPAT. A_e contracted with 3×10^{-5} *M* DPAT. $C_{a,b}$ contracted with 6×10^{-5} *M* DOI. The tension bar, in g represents 0.25 g for A_{e-f} and B_c; 0.5 g for $B_{a,b}$ and; 0.125 g for B_d and $C_{a,b}$. Additions of ATP are represented by 0.18 and 1.8 as μM concentrations. Additions of αβ-methylene ATP are represented by 0.2 and 2 as μM concentrations. Acetylcholine (Ach) additions are 0.3 μM for A_a and 6 μM for B_a.

bars in the figures—represent individual data points obtained with different tissue preparations. In all cases a *P* value of less than 0.05 was taken as significant.

Results

Serotonin- and DPAT-Induced Arterial Contractions. Initially, studies were conducted to determine if the serotonin-induced contraction of rat pulmonary arterial ring segments was dependent upon selective 5HT receptor subtypes. Figure 1 demonstrates that a $5HT_{1A}$ agonist, DPAT [(+)-8-hydroxy-2-(di-*N*-propylamino)tetralin hydrobromide], effectively began to induce arterial contraction at concentrations of approximately 10^{-6} *M*. However, 5HT was a more efficacious agonist than DPAT at all comparable concentrations measured, with approximately 10-fold less agonist required to achieve similar levels of contraction (at least nine determinations, each with different tissue preparations, were performed at each concentration between 3×10^{-7} and 6×10^{-5} *M*). The level of contraction induced by the two agonists was significantly different, as determined by a Student's *t* test, at all concentrations tested. The $5HT_{1C}$ and $5HT_2$ agonist, DOI [(±)-2,5-dimethoxy-4-iodoamphetamine hydrobromide], was even less effective than DPAT, inducing contraction only at concentrations exceeding 10^{-5} *M*. A study, performed in duplicate, with the $5HT_3$ agonist, 1-phenylbiguanide, did not induce arterial contractions at concentrations up to 9×10^{-5} *M* (data not shown).

Inhibitors with varying 5HT receptor type selectivity were employed in an attempt to substantiate the involvement of a $5HT_2$-type–induced vasoconstriction (Fig. 2). Inhibition of 5HT-induced contraction by the $5HT_2$ antagonist, ketanserin, was observed with an IC_{50} of 2.4×10^{-7} *M*. The antagonists, propranolol and metoclopramide, with limited selectivity for $5HT_1$ and $5HT_3$, respectively, inhibited the 5HT-induced contraction approximately equally with an IC_{50} of $2–3 \times 10^{-5}$ *M*. These were 100-fold less sensitive than ketanserin.

ATP and αβATP Modulation of Serotonin-Induced Arterial Contractions. ATP and its poorly hydrolyzable analog, αβATP, were added alone and after the addition of serotonin or serotonin analog to the arterial rings to assess their potential to modulate the serotonin-induced contraction. ATP and αβATP alone, at the concentrations employed, did not significantly alter the arterial tone. The concentration of DPAT employed, 60 μM, was twice that of 5HT due to its weaker ability to induce arterial contractions. In each case the concentration employed was approximately the EC_{50}, and within the limits of the concentrations studied gave nearly equal contractions. ATP at 0.18 and 1.8 μM relaxed the 5HT and DPAT contracted tissue approximately equally, as depicted in Table I. A representative tracing of the ATP-induced relaxation is shown in Figure 3, panel A. The ATP-induced relaxation of the serotonin-contracted tissue required an interaction with the endothelium (Fig. 3B).

Unlike ATP, αβATP further increased the level of 5HT-induced arterial contraction. The relative αβATP effect was significantly greater with the DPAT-contracted tissue than with the 5HT-contracted muscle (Table I and Fig. 3, Panel A). As can be seen from Table I. the 2 μM αβATP-induced additional contractions of the serotonin-contracted tissues were very variable from preparation to preparation. Only four out of the many tissue preparations employed in this series of studies analyzed the effect of 2 μM αβATP with both 5HT and DPAT. When the effect of αβATP on 5HT- and DPAT-induced contractions in these four tissue samples was analyzed by a paired Student's *t* test, the *P* value was <0.05. This concentration of αβATP alone did not cause tissue contraction and was also dependent upon the presence of endothelium (Fig. 3B). In tissue that responded minimally (<0.12 g tension) to DPAT or DOI, a very large, but highly variable, contractive response

Table II. The Percent Contraction/Relaxation of Rat Pulmonary Artery Rings Relative to Control KCl Contracted Tissue

	Final % contraction
30 μ*M* 5HT	49.3 ± 5.2 (10)
30 μ*M* 5HT → 18 μ*M* ATP	24.3 ± 4.2 (10)
18 μ*M* ATP	2.3 ± 0.8 (10)
18 μ*M* ATP → 30 μ*M* 5HT	24.1 ± 3.8 (10)
18 μ*M* ATP + 30 μ*M* 5HT	23.5 ± 4.2 (10)

Note. Data are presented as in Table I.

was usually observed with the subsequent addition of αβATP (Fig. 3, A, e and C, b). ATP, like αβATP, synergistically contracted the DOI-treated tissue, in contrast to the relaxant effect of ATP observed with 5HT- and DPAT-contracted arterial segments (Fig. 3C). Higher concentrations of DPAT and DOI were employed for these studies based upon the results, depicted in Figure 1, that demonstrated lower sensitivities compared with 5HT.

The order in which the rat pulmonary arterial rings were exposed to 5HT and ATP was varied to determine if the net response would be altered. The results, presented in Table II, demonstrate that the final level of vessel contraction was the same whether ATP was added prior to, after, or simultaneously with 5HT. When ATP was added to the 5HT-contracted tissue, it relaxed the tissue (24.3%) to a final contracted state of 25% of the KCl control level. This was statistically the same as the final contracted state under the other two conditions depicted in Table II. Figure 4 also demonstrates that the addition of 18 μ*M* ATP to the tissue bath prior to the titration with 5HT significantly (<0.05) inhibited contraction at all concentrations of 5HT except at the two lowest concentrations.

ATP and ATP Analogs. The differential effects of ATP and αβATP indicated that more than one P_2 receptor type was present in the rat arterial ring segment. ATP or one of several different ATP analogs was therefore added simultaneously with 5HT to determine if a specific purinoceptor subtype was associated with the ATP modulation of 5HT-induced contractions. The results of a representative experiment are depicted in Figure 5 and again reflect the differential effects of ATP and αβATP. While the P_{2x} agonist, αβATP, in conjunction with 5HT, induced a greater tissue contraction than 5HT alone, ATP and the partial P_{2y} agonist, BzATP (3′-*O*-[4-benzoyl]benzoyl ATP) (34), opposed the contractive effect of 5HT. Inexplicably, the P_{2x} agonist, βγATP, consistently behaved like ATP and BzATP and not the αβATP analog.

cAMP. We previously demonstrated that ATP inhibition of platelet function appeared to correlate with ATP stimulation of cAMP levels (17, 18). A series of experiments were therefore conducted with the phosphodiesterase inhibitor, theophylline, to determine if increased levels of cAMP would impact on the cumulative activities of 5HT or ATP. If ATP in the arterial tissue functioned in a similar fashion to that observed with platelets, the further stimulation of cAMP levels

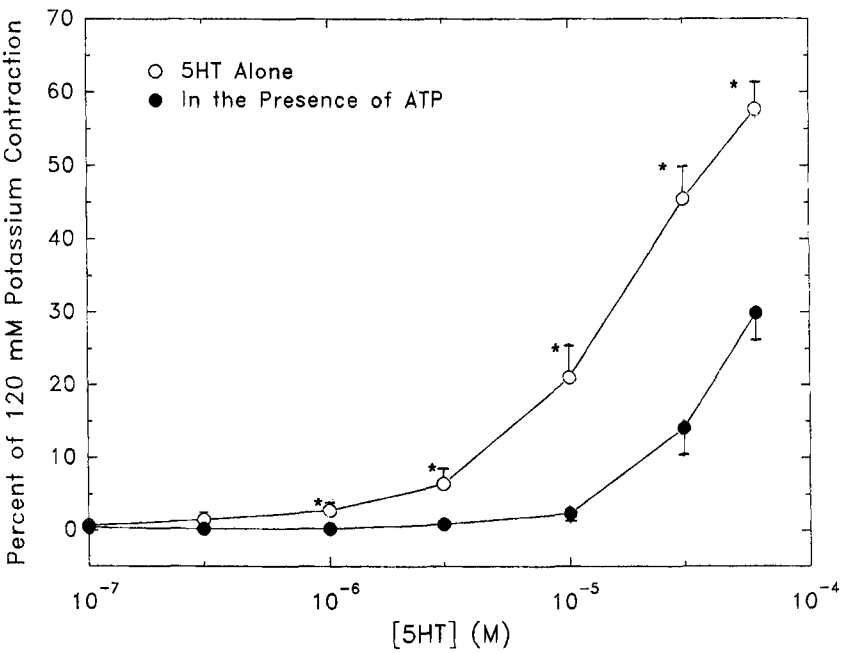

Figure 4. Serotonin-induced contractions of rat pulmonary arterial rings with increasing concentrations of 5HT in the presence or absence of 18 μ*M* ATP. The contractions attained at all levels of 5HT except at 10^{-7} *M* were significantly reduced by the presence of ATP. Each point is the mean of six experiments; bars, SEM; $^*P < 0.05$.

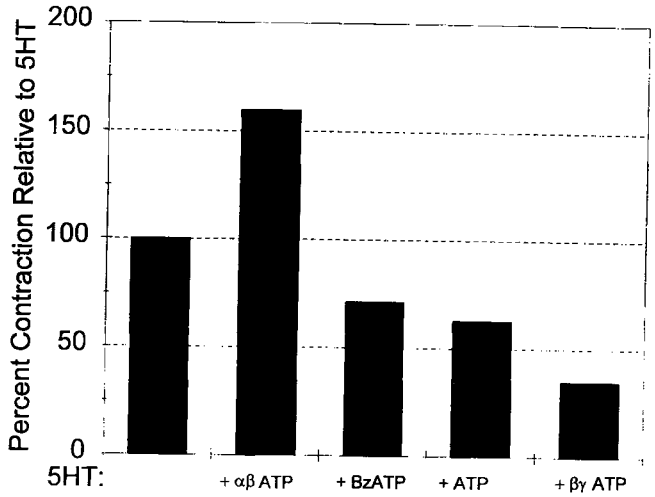

Figure 5. Serotonin-induced contractions of rat pulmonary arterial rings in the absence or presence of ATP or ATP analogs. The level of tissue contraction was compared to the 30 μ*M* serotonin-induced contraction which was normalized to 100%. The concentration of ATP was 18 μ*M* while that of αβATP, βγATP, and BzATP were all 20 μ*M*.

would, it was reasoned, further enhance the ATP inhibition/relaxation of 5HT-induced vasocontraction. The antagonistic activity of theophylline on the adenosine receptor should have no role in these studies. Initial studies demonstrated that theophylline itself inhibited 5HT-induced vasoconstrictions. A theophylline concentration that inhibited the 5HT-induced contraction approximately 50% was therefore chosen. The tissue bath was adjusted to 0.5 m*M* theophylline, and then the intact (Fig. 6A) or denuded (Fig. 6B) arterial rings were titrated with increasing amounts of 5HT, in the presence or absence of 18 μ*M* ATP. The results demonstrated that theophylline inhibited the 5HT-induced contraction (compare the level of contraction in Fig. 6 with that in Fig. 4), the inhibition was independent of endothelial cells, and the relaxing/inhibitory effect of ATP (seen only with intact tissue) was neither enhanced nor abrogated by theophylline. The percent relaxing/inhibitory effect of ATP remained approximately the same in the presence or absence of theophylline (compare Fig. 4 with Fig. 6A).

The (PGI_2) analog, iloprost (a gift from Berlex Laboratories, Wayne, NJ), was also tested since it was reported to increase greatly intracellular cAMP levels (17). In a limited number of studies, it consistently inhibited 5HT-induced contractions as did theophylline (data not shown).

Discussion

Serotonin (5HT) and ATP are two members of a complex multicomponent interactive system that regulate vascular tone and hemostasis. While it is well established that 5HT and ATP can individually modulate vascular tone, no work, save our preliminary study (11), has investigated the effect of these two components added together. Therefore, this study focused on the vasoactive interactions between 5HT and ATP, which are simultaneously released, at high concentrations, from activated platelets within localized regions of vascular injury. Platelet-released ATP, as well as ATP derived from other sources, can modulate platelet function by inhibiting agonist-induced aggregation of platelets (17, 18). This inhibition would reduce the availability of released vasoactive compounds and regulate the size of the resulting clot. ATP and 5HT can also regulate regional vascular tone (11, 20) that would modulate vascular geometry, flow rates, and shear forces impacting on hemostasis and thrombosis (1). The elucidation of 5HT and ATP receptor types available in specific

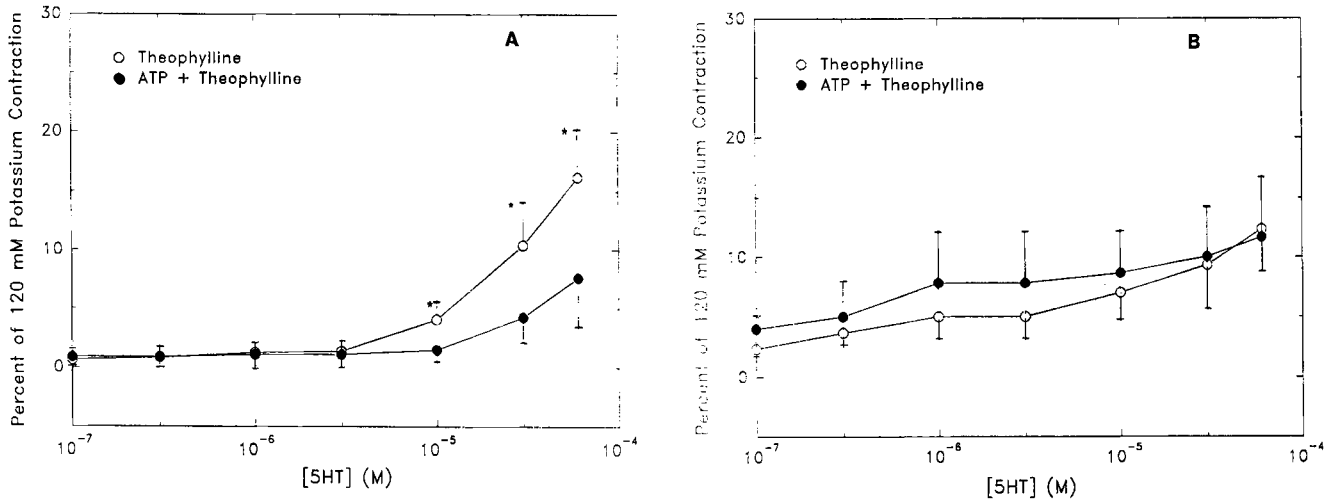

Figure 6. Serotonin-induced contractions of 0.5 m*M* theophylline treated rat pulmonary arterial rings with increasing concentrations of 5HT in the presence or absence of 18 μ*M* ATP. (A) Contractions of intact tissue. (B) Contractions of de-endothelialized tissues. Each point in Panel A is the mean of five determinations and in Panel B is the mean of three determinations; bars, SEM. Only the last three points of 5HT plus ATP (*) in Panel A were significantly different from the tissue treated with 5HT alone ($P < 0.05$).

blood vessels and how their ligand occupancy regulates regional vascular tone may facilitate the development of efficacious anti-thrombotic drugs.

Our results imply the presence of both $5HT_2$ and $5HT_1$ receptors in rat pulmonary arteries, as has been reported in bovine (35) and human (36) coronary arteries. DPAT is a selective $5HT_{1A}$ agonist at concentrations 1000-fold less than those employed in these studies (15). The higher levels of DPAT required to induce contractions of rat pulmonary arterial segments in our studies may reflect the presence of the 5HT receptor subtypes 1B, 1C, and/or 1D. Similar responses were observed in canine coronary arterial segments with DPAT when the relaxing effects of the endothelium were subtracted (37). Also, Foy *et al.* (35) observed similar responses to 5HT and DPAT in bovine coronary arteries with inhibition by propranolol (2.6 μM) and ketanserin (0.3 μM), as in our study. Houston and Vanhoutte (37) concluded that while some $5HT_2$ sites may be present in their tissue preparations, other $5HT_1$ receptor types appeared to predominate. However, it should be noted that ketanserin at 10^{-6} M in their study gave only slight inhibition, while in our study nearly 10-fold less ketanserin inhibited 5HT-induced contractions with an IC_{50} of 2.4×10^{-7} M.

The concentrations of 5HT required to induce contraction may reflect the presence of both $5HT_1$- and $5HT_2$-type receptors on the smooth muscle cells. The concentration of ketanserin required to inhibit 5HT receptors in our studies was 5- to 50-fold higher than normally employed to inhibit $5HT_2$ receptors and could indicate that the antagonist is interacting with $5HT_{1C}$ and $5HT_{1D}$ receptors (13, 15), as well as $5HT_2$ receptors. It may also be that higher concentrations of ketanserin are required to displace 5HT under our experimental conditions. Evidence for the presence of $5HT_1$ receptor types on the rat pulmonary artery is also indicated by the antagonistic response to (±) propranolol. The IC_{50} in our studies was only about 10-fold less than that observed for $5HT_1$ and $5HT_{1B}$ receptor types in rat frontal cortex slices (33), and the difference is reduced further when one takes into consideration the fact that we employed the mixed stereoisomeric forms (±) while the (+) form is essentially inactive (33). The lack of activity observed with DOI is inconsistent with the presence of either $5HT_2$ or $5HT_{1C}$ sites (15). This is particularly surprising since these are the two major sites antagonized by ketanserin (15). Our results may reflect an absence of $5HT_2$ receptors or an inability of DOI to act in the rat system rather than a lack of receptor types. Further work is required to definitively identify the 5HT receptor types present in rat pulmonary arteries. Short of cloning the 5HT receptor gene(s) expressed in this tissue, their exact identity is problematic. However, our study, along with data compiled by others, indicates the presence of more than one 5HT receptor type in this tissue.

Further evidence for the presence of more than one 5HT receptor type in rat pulmonary arteries comes from the differential behavior of 5HT-contracted tissue with ATP and αβATP. We have shown, here and previously, that ATP relaxes and αβATP further contracts 5HT-contracted rat pulmonary arteries (11). Others have similarly demonstrated that low concentrations of ATP (1 nM–1 μM) relax and αβATP contract human omentum resistance arteries contracted with noradrenaline (38). Unlike Martin *et al.* (38), we did not detect any significant contraction by ATP alone at concentrations above 1 μM. The ability of ATP to relax 5HT-contracted arterial segments was the same with 5HT- and DPAT-treated tissue and was endothelium dependent. This would imply an interaction of ATP with endothelial P_{2y} receptors and the release of endothelial-derived relaxing factor (39). ATP, however, induced vasoconstriction of DOI-treated tissue in a synergistic fashion, indicating the presence of a $5HT_x$-P_2 interactive response distinct from that observed with 5HT and DPAT. This occurred even in the absence of a detectable DOI-induced contractive response. Finally, DPAT-contracted tissue was further contracted by αβATP to a significantly greater extent than that observed with 5HT-contracted tissue. The αβATP response was also entirely endothelium dependent as opposed to dependent and independent responses observed with canine arteries (40) and may result from the release of an endothelial-derived contracting factor.

Endothelial cells contain P_{2y} receptors, while smooth muscle cells have P_{2x} and in some instances P_{2y} receptors (20, 39). Our results with ATP and BzATP conformed with expectations where 5HT-induced contractions were relaxed/inhibited *via* interactions with endothelial P_{2y} receptors and the subsequent release of endothelial relaxing factor. This was further substantiated by the abrogation of the ATP effect with denuded arterial rings. The results with the two P_{2x} agonists, αβ ATP and βγATP, did not readily fit the expected patterns. αβATP consistently enhanced serotonin contractions, presumably by interactions with the smooth muscle P_{2x} receptors (41). However, this response was lost upon removal of the endothelium. The βγATP analog relaxed rather than enhanced 5HT-induced contractions. These apparently aberrant responses may be due to (i) a different interaction with an endothelial factor(s); (ii) differences in the ability of the analogs to reach the P_2 receptors; (iii) differential hydrolysis by ectonucleotidases; or (iv) the unique distribution of P_{2x}, P_{2y}, or other P_2 receptors in this tissue. Further studies are required to resolve these issues.

These studies indicated for the first time that theophylline inhibits 5HT-induced contractions presumably due to an increase of intracellular cAMP levels.

This is consistent with an antagonistic response to a $5HT_{1\text{-like}}$ receptor response which is coupled to G_i proteins that inhibit adenyl cyclase (42, 43). The $5HT_2$ receptors, on the other hand, are coupled to G proteins that activate phosphoinositide metabolism (43). It is not apparent, at this time, whether or not the $5HT_2$-induced contraction would also be affected by theophylline-induced increases of cAMP. The inhibitory effect was endothelium independent and had no apparent effect on the ability of ATP to relax the 5HT-contracted tissue. This was in sharp contrast to the synergistic effect of theophylline and ATP on platelet function (17). While we have previously shown that ATP enhances the cAMP levels in platelets (17, 18), it would appear from the additive relaxing/inhibitory effects of ATP and theophylline that ATP acts *via* a different mechanism in this system. P_{2u}/P_{2y} receptors are coupled to G proteins that regulate the PI/PLC pathways (2), which may be associated with the ATP effect we observed with the rat tissue. It is possible that the vasodilating action of theophylline (44) is related in part to its ability to inhibit 5HT-induced contractions via its inhibition of phosphodiesterase and is independent of its adenosine antagonistic properties (39).

Blood vessels at sites of injury may be exposed to ATP and 5HT with varying sequences of delivery of these two agents. Hypoxia may initially induce tissue damage with release of ATP followed by activation of platelets at the lesion site and subsequent release of 5HT plus ATP. ATP and 5HT would be released from the platelets simultaneously at the site of injured blood vessels. Finally, hypoxia may follow release of platelet 5HT and ATP if the damaged blood vessel becomes occluded, with the further release of tissue ATP. The response of the rat pulmonary artery to 5HT and ATP appeared to be independent of the order in which it was exposed to these agents. In all cases, the contractive effect of 5HT was reversed or inhibited by ATP to the same extent. The net effect of ATP was to relax or inhibit the potential occlusion of the blood vessel by 5HT.

The distribution of 5HT and ATP receptor types within the cardiovascular system appears to vary from tissue to tissue and from species to species. The simultaneous presentation of 5HT and ATP to different vascular regions can, therefore, result in vasoconstrictive or vasodilatory responses which would impact on the hemostatic process. One would imagine that, under normal hemostatic processes, these apparently opposing effects serve a beneficial regulatory role and reflect localized requirements dependent upon vessel size, function, and geometry. Under pathological conditions, alterations in the balance of these localized receptor types may have catastrophic consequences, as may occur in atherosclerosis (5, 6). A thorough knowledge of the vascular interactions of 5HT and ATP would facilitate the selection of drugs to be developed and employed to treat pathological conditions of the cardiovascular system.

Our thanks to Michelle Soslau for stimulating and helpful comments and to Dr. Robert McKenzie for his assistance in the presentation of data.

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