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Renal vascular reactivity to ATP in hyper- and hypothyroid rats

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Abstract. The effects of adenosine triphosphate (ATP) on the renal vasculature of isolated kidneys from control, hyper- and hypothyroid rats were characterized. ATP responsiveness was evaluated in basal tone and in raised tone (phenylephrine 10^{-6} M) preparations. These responses were compared with those obtained with barium chloride or sodium nitroprusside (SNP), used respectively as nonreceptor agonists for vasoconstriction or vasodilation. In preparations at basal tone, ATP produced dose-related vasoconstriction, which was increased in hyperthyroid kidneys, and was severely attenuated in kidneys from hypothyroid rats. In raised tone preparations from control rats ATP produced a dual response: vasoconstriction at low doses, which declined with increasing doses to give way to vasodilator responses; biphasic responses were found in some kidneys. Hyperthroid kidneys showed increased pressor responses and a vasodilator response similar to those seen in kidneys from control rats. However, in hypothyroid kidneys the vasodilator response was abolished. The responses to barium chloride and to SNP were significantly increased and decreased in hyper- and hypothyroid kidneys, respectively; vasoconstrictor responses to SNP were also found in hypothyroid kidneys. Hence the abnormal responses to ATP observed in both thyroid dysfunctions may be partially explained by unspecific alterations in the contractile machinery of the renal vasculature in these kidneys. However, ATP responsiveness (vasoconstriction at low tone and vasodilation at raised tone) was more severely affected in hypothyroid kidneys, suggesting that purinergic (P_{2X} and P_{2Y}) receptor activity may be decreased in these organs.
Key words. Hyperthyroidism; hypothyroidism; vasodilation; vasoconstriction; adenosine triphosphate (ATP); isolated kidney.

The actions of adenosine triphosphate (ATP) on cardiac function, platelets, mast and endothelial cells can influence the cardiovascular system under physiological and pathophysiological conditions[1]. Moreover, there is evidence of cotransmission of noradrenaline (NA) and ATP in sympathetic nerves[2], and a synergistic interaction between ATP and NA has been described in different preparations[3,4]. ATP may have a dual function in the regulation of vascular tone: 1) as a vasoconstrictor acting via excitatory P_{2X}-purinoceptors in low tone preparations, and 2) as a vasodilator acting via inhibitory P_{2Y}-purinoceptors in raised tone preparations[5]. The clinical manifestations of hyperthyroidism resemble those of sympathetic nervous systems activation. There is hyperdynamic circulation characterized by increased cardiac output, diminished peripheral vascular resistance and increased blood pressure[6,7]. The opposite clinical findings are found in hypothyroidism[6,7]. Thyroid disorders are also accompanied by changes in peripheral adrenergic activity[8,9] produced by altered adrenergic receptor densities in many tissues[10–12]. The alterations in the adrenergic system may be associated with changes in the activity of the purinergic component of sympathetic transmisssion. Therefore, it is plausible to postulate that the purinergic system may be affected by pathological thyroid functioning.

In the present study we examined the constrictor and relaxant response to ATP in the renal vascular bed of rats with chronic (6 week) hyper- or hypothyroidism. Because nonspecific alterations in the responsiveness to vasoactive agents were found in throyid disorders[13], these responses were compared with those obtained after the administration of nonreceptor-mediated vasoconstrictor and vasodilator agonists.

Material and methods

Male Wistar rats initially weighing 180–200 g were maintained on standard chow and tap water ad libitum except where stated. The animals were divided into three groups: control, hyperthyroid, and hypothyroid (n = 15, each group). Hyperthyroidism was induced by injecting thyroxine 300 μg/kg/day s.c. dissolved in 0.5 N NaOH isotonic saline. Hypothyroidism was induced by the continuous administration of 0.03% methimazole via drinking water. Both treatments were administered for six weeks. The effectiveness of these treatments was

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assessed by comparing the final thyroid, renal, and body weights, and serum thyroxine (T_4) and triiodothyronine (T_3) in control and treated rats. Serum thyroid hormone levels were determined by ELISA (Immunoassay System, Baxter, Miami, USA).

Rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.). The abdomen was opened by a midline incision and the left renal and superior mesenteric arteries exposed. To maintain continuous renal perfusion, the superior mesenteric artery was cannulated with a beveled, 18-gauge needle that was advanced into the left renal artery and secured with ligatures. The kidney was perfused at a constant flow [5 ml/g of kidney weight (kw) per minute] by means of a roller pump (IPS-4, Ismatec S.A., Zürich, Switzerland), with Tyrode solution (37 °C). The composition of Tyrode solution in mM was: NaCl, 137; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 1.1; $NaHCO_3$, 12.0; NaH_2PO_4, 0.42; D(+) glucose, 5.6 aerated with 5% CO_2 in O_2. The kidney was then excised from its surrounding tissues and placed in a chamber containing Tyrode solution at 37 °C. Renal vascular responses were recorded (TRA-021 transducer connected to a two-channel Letigraph 2000 recorder, Letica S.A., Barcelona) as changes in renal perfusion pressure (RPP) downstream from the pump.

After 30 min of equilibration, dose-response curves to ATP (10^{-6} to 10^{-5} M/gkw) and barium chloride (10^{-6} to $5 \cdot 10^{-5}$ M/gkw) were obtained at basal (low) tone (n = 6, for each group). The agonists were rapidly injected in constant volumes (50 µl/gkw for ATP and 100 µl/gkw for barium chloride) into the perfusion system, close to the kidney. Injection of these volumes caused a small, transient increase in RPP which preceded the agonist-evoked response. After 30 min of equilibration, other kidneys (n = 6 each group) were preconstricted (raised tone) with 10^{-6} M phenylephrine; dose-response curves to ATP (10^{-10} to 10^{-7} M/gkw) and sodium nitroprusside (SNP) (10^{-10} to 10^{-6} M/gkw) were then obtained (50 µl/gkw). Because renal vascular reactivity to phenylephrine is increased in hyperthyroid and decreased in hypothyroid rats[13], the dose of phenylephrine infused was adjusted in hypo- and hyperthyroid kidneys to produce a similar increase in the vascular tone. Before commencing the experiment, a stabilization period of 30 min in the presence of indomethacin (10^{-5} M) was allowed to inhibit the release of prostaglandins induced by ATP. All ATP experiments (low and raised tone) were performed in the presence of 8-phenyltheophylline (10^{-5} M), a P_1 receptor antagonist, to prevent the possible effect of adenosine generated by the breakdown of ATP. This dose of 8-phenyltheophylline did not significantly modify the dose-response curve to $BaCl_2$ or NP in preliminary experiments (data not shown). The minimum time between successive doses of agonist (vasoconstrictors and vasodilators) was 5 min. When necessary, these periods were extended until the previous response had disappeared. The minimum period between agonists was 15 min. Changes in RPP in response to vasodilators were expressed as the percent of vasoconstriction obtained with phenylephrine. Doses of agonists were adjusted per gram of kidney weight (contralateral kidney).

The following drugs were used: heparin (Leo, Madrid, Spain), pentobarbital sodium (Nembutal, Serva, Heidelberg, Germany), thyroxine (Merck, Darmstadt, Germany), methimazole, adenosine 5′-triphosphate, sodium nitroprusside, 8-phenyltheophylline, indomethacin, phenylephrine hydrochloride (Sigma).

One-way ANOVA analysis was carried out to compare dose-response curves between groups at each dose and biological variables. When the differences were significant, the Bonferroni method was used for subsequent comparisons.

Results and discussion

The effects of thyroxine of methimazole administration on biological variables are presented in the table. Animals given thyroxine or methimazole for six weeks gained significantly less weight than their age-matched controls during this period. Renal weight, serum T_3 and T_4 levels were decreased and increased in hypo- and hyperthyroid rats, respectively. Thyroid weight was increased in hypothroid and decreased in hyperthyroid rats. Hence rats given methimazole for six weeks developed the characteristic manifestations of hypothyroidism, whereas those given thyroxine for a similar period developed hyperthyroidism.

Basal RPP was similar in all three experimental groups (control = 43 ± 2.5 mmHg; hyperthyroid = 49.5 ± 5.0 mmHg; hypothyroid = 52.5 ± 5.5 mmHg). In preparations at basal tone (fig. 1), ATP elicited dose-dependent contractions in the renal vasculature from control rats. In hyperthyroid kidneys, this response was

Table. Biological variables. Body weight (BW), renal weight (RW), thyroid weight (TW) and plasma concentrations of thyroxine (T_4) and triiodothyronine (T_3) in control (C), hyperthyroid (HE, treated with thyroxine 300 µg/kg/day, s.c.) and hypothyroid (HO, treated with 0.03% methimazole in the drinking water) rats.

Groups	BW (g)	RW (mg)	TW (mg)	T_4 (µg/dl)	T_3 (ng/dl)
C (n = 6)	425.0 ±8.1	1256 ±62	34.7 ±2.1	3.7 ±0.17	53 ±8.3
HE (n = 6)	238.5** ±14.2	1675* ±51	22.4* ±1.7	40.7** ±0.17	345** ±7.2
HO (n = 6)	261.1** ±3.73	851* ±51	166* ±12.7	0.23** ±0.2	4** ±3.1

Data are mean ± SEM, *p < 0.05, **p < 0.01 compared with control group.

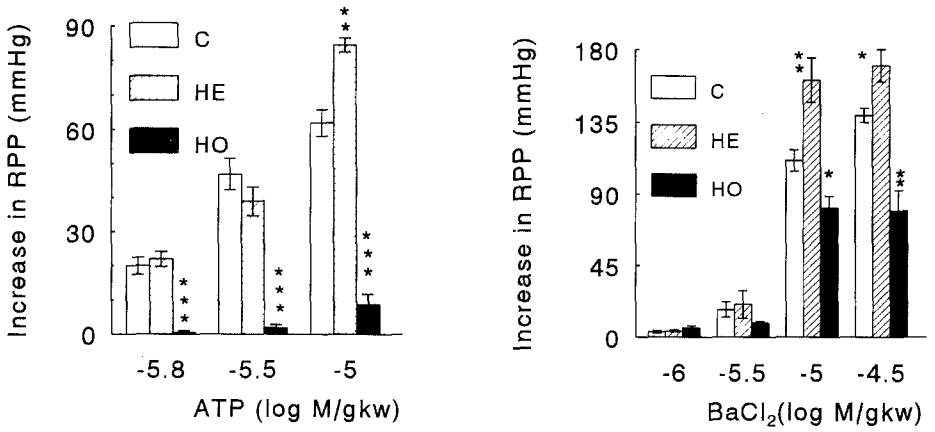

Figure 1. Absolute increase in renal perfusion pressure (RPP) produced by ATP or barium chloride in isolated perfused kidneys, at basal tone, from control (C), thyroxine-treated (hyperthyroid, HE) and methimazole-treated (hypothyroid, HO) rats (r = 6 each group). Doses of agonists were adjusted per gram of kidney weight (contralateral kidney). *p < 0.05, **p < 0.01; ***p < 0.001 compared with control group. Data are means ± SEM.

cantly increased at the highest dose. However, the dose-response curve of hypothyroid kidneys was blunted, with only a modest increase in RPP at the highest dose. The response to barium chloride was also increased in hyperthyroid and decreased in hypothyroid kidneys. However, although the response to barium chloride was significantly reduced in hypothyroid rats, it was not as severely attenuated as the response to ATP.

In preconstricted renal vasculature (phenylephrine 10^{-6} M) from control rats, ATP at low doses elicited vasoconstrictor responses, followed by vasodilator responses with increasing doses (fig. 2). Biphasic responses to ATP (contraction followed by relaxation) were also observed in the preconstricted renal vasculature from hyperthyroid rats. In hyperthyroid kidneys, the vasoconstrictor and the vasodilator responses were not significantly different from those observed in the control group. However, in hypothyroid kidneys, the vasodilator response was suppressed and vasoconstrictor responses occurred even at high doses. SNP produced a dose-related vasodilatation in control kidneys. This response was significantly increased in hyperthyroid kidneys. This response was significantly increased in hyperthyroid kidneys. However, hypothyroid kidneys showed a dual response: vasoconstriction at low doses and vasodilation at high doses. The vasodilator responses were similar to those observed in control kidneys.

The results of the present study demonstrate that ATP, at basal tone, causes dose-dependent vasoconstriction in the rat renal vasculature, as previously reported for other arterial beds[14,15]. However, in the preconstricted renal vascular bed a dual response to ATP is observed depending on the dose of agonist. Thus, low doses of ATP induced vasoconstriction, which weakened with increasing doses to give way to vasodilation. Moreover,

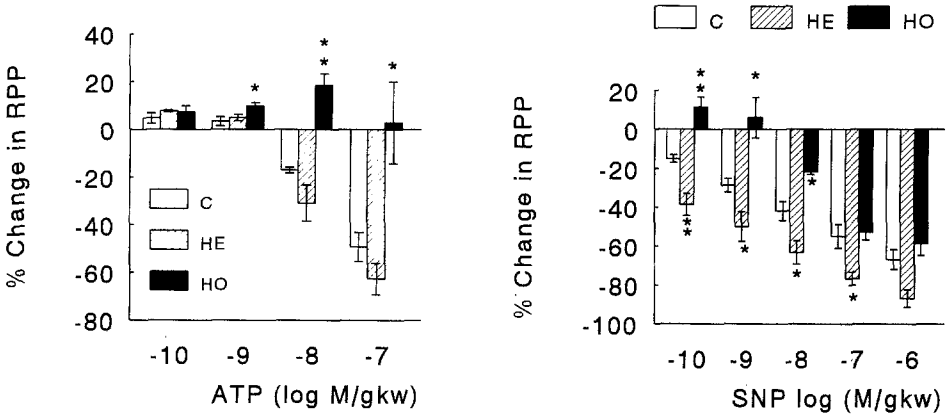

Figure 2. Effect of increasing bolus doses of ATP or sodium nitroprusside (SNP) on renal perfusion pressure (RPP) of phenylephrine-preconstricted isolated perfused kidneys from control (C), thyroxine-treated (hypothyroid, HO) rats (r = 6, each group). RPP before commencing with the dose-response curves were: C = 120 ± 5 mmHg; HE = 127 ± 6 mmHg; HO = 118 ± 3 mmHg.
*p < 0.05; **p < 0.01 compared with control group. Doses of agonists were adjusted per gram of kidney weight (contralateral kidney). Data are means ± SEM.

biphasic responses consisting of an initial contraction followed by relaxation were also found in the preconstricted renal vascular bed of control rats. These findings are compatible with the response of precontracted smooth muscle of the mouse vas deferens[16] and with similar biphasic effects in other vascular preparations[17,18]. The dual response to ATP in the preconstricted renal vascular bed is in sharp contrast to the exclusively vasodilator response observed in isolated mesenteric[15] and hepatic[19] vascular beds. These discrepancies may be due to the specific characteristics of the renal vasculature, or to the presence of indomethacin, as ATP and ADP stimulate the release of prostaglandins from vascular beds[20,21].

It has been proposed that the contracting and relaxing effects of ATP are mediated through different receptors (P_{2X} and P_{2Y}). Thus, vasoconstriction under basal conditions or in preconstricted vasculature is produced by the activation of P_{2X}-purinoceptors, whereas vasodilation is produced by the activation of P_{2Y}-purinoceptors[1]. Hence the results of the present study confirm previously reported data[22] indicating the presence of both P_2 purinoceptors in the renal vasculature.

In hyperthyroid kidneys, ATP at low tone produced a greater increase in RPP than that observed in control rats; however, the vasodilator response was similar to that found in controls. These data suggest that in hyperthyroidism there is an increase in the number of renal vasoconstrictor P_{2X} receptors without changes in the vasodilator P_{2Y} purinoceptors. However, renal responsiveness to barium chloride was also increased in these kidneys, indicating that augmented responsiveness to ATP may be due to nonspecific alterations in the contractile machinery of the renal vasculature in these rats. In contrast, the vasoconstrictor response to ATP in hypothyroid kidneys at low tone was almost absent, and relaxation in the preconstricted vasculature was also markedly attenuated. Although vasoconstriction and vasodilation induced by nonreceptor agonists in hypothyroid kidneys were significantly modified, both ATP-induced responses (vasoconstriction and vasodilatation) were more severely affected than those observed when nonreceptor agonists and other receptor-mediated vasodilators (acetylcholine) or vasoconstrictors (phenylephrine, vasopressin) were used[13]. Hence, the reduced responsiveness to both actions of ATP in hyper- and hypothyroid kidneys cannot be totally explained by nonspecific alterations in renal vascular smooth muscle cells. Thus, our results suggest that hypothyroidism reduced both P_{2X} and P_{2Y} purinoceptors. These results also indicate that other experimental approaches are needed, such as a study of binding of these receptors to determine directly the influence of thyroid disorders on P_2 receptors. However, these studies cannot be performed in the renal vasculature due to obvious difficulties in the separation of renal vasculature. Because of these difficulties we have changed the preparation and used the mesenteric vasculature instead. In this preparation we have determined the activity of both P_2 receptors in thyroid disorders, using specific agonists in order to establish possible correlations with future binding studies. These studies in the mesenteric vasculature showed reduced P_{2X} activity in preparations from hypothyroid rats and increased activity in preparations from hyperthyroid rats (unpubl. observations), data that agree with the results reported in the present paper.

It is also interesting to note that the bolus administration of SNP produced a dual response in preconstricted renal vasculature of hypothyroid kidneys: vasoconstriction at low doses and vasodilation at high doses. This finding is compatible with data obtained in our laboratory with kidneys from aged rats[23]. We have no explanation for these unexpected observations, and additional studies will be necessary to elucidate the mechanism by which SNP produces vasoconstriction in the renal vasculature under these circumstances.

In summary, our results demonstrate that hyperthyroid kidneys show increased responsiveness to the vasoconstrictor effect of ATP, whereas in hypothyroid kidneys the vasoconstrictor response is almost completely suppressed and the vasodilating effect of ATP is reduced. Although these changes may be partially due to nonspecific alterations of the vascular smooth muscle produced by the thyroid dysfunctions, the possible contribution of a decreased activity of P_{2X} and P_{2Y} purinoceptors in hypothyroid kidneys is also suggested.

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