Local regulation of vasopressin and oxytocin secretion by extracellular ATP in the isolated posterior lobe of the rat hypophysis

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Abstract

It is now widely accepted that ATP functions as a signalling substance in the nervous system. The presence of P_2 receptors mediating the action of extracellular ATP in brain regions involved in hormonal regulation raises the possibility that a similar role for ATP might also exist in the neuroendocrine system. In this study, the release from the rat isolated neurohypophysis preparation of endogenous ATP, oxytocin and vasopressin (AVP) were measured simultaneously using luciferin–luciferase and RIA techniques. After 70 min preperfusion, electrical field stimulation caused a rapid increase in the amount of ATP in the effluent and the release of AVP and oxytocin also increased stimulation-dependently. Inhibition of voltage-dependent Na^+ channels by tetrodotoxin (1 µM) reduced the stimulation-evoked release of AVP and oxytocin; however, the evoked release of ATP remained unaffected.

The effect of endogenous ATP on the hormone secretion was tested by suramin (300 µM), the P_2 receptor antagonist. Suramin significantly increased the release of

AVP, and the release of oxytocin was also enhanced. ATP, when applied to the superfusing medium, decreased the release of AVP, but not that of oxytocin, and its effect was prevented by suramin.

ATP (60 nmol), added to the tissues, was readily decomposed to ADP, AMP and adenosine measured by HPLC combined with ultraviolet light detection, and the kinetic parameters of the enzymes responsible for inactivation of ATP (ectoATPase and ecto5′-nucleotidase) were also determined ($K_m = 264 \pm 2·7$ and 334 ± 165 µM and $v_{max} = 6·7 \pm 1·1$ and $2·54 \pm 0·24$ nmol/min per preparation ($n=3$) for ectoATPase and ecto5′-nucleotidase respectively).

Taken together, our data demonstrate the stimulation-dependent release, P_2 receptor-mediated action and extracellular metabolism of endogenous ATP in the posterior lobe of the hypophysis and indicate its role, as a paracrine regulator, in the local control of hormone secretion.

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Introduction

Although the function of ATP as a ubiquitous extracellular signalling substance has been gaining an increasing body of experimental support for decades (Drury & Szent-Györgyi 1929, cf. Burnstock 1997), it has only recently been recognized that it also acts as a neuroendocrine regulatory messenger (Chen *et al.* 1995b). Subtypes of the P_2 purinoceptor superfamily mediating the action of extracellular ATP are widely distributed in neural and non-neuronal tissues, and they are also expressed in organs involved in endocrine regulation, including the pituitary (Brake *et al.* 1994, Chen *et al.* 1995b, North 1996). Until now, research interest in this field has concentrated on the anterior lobe of the pituitary gland, where both the exocytotic release of endogenous ATP (Chen *et al.* 1995a, Tomic *et al.* 1996) and its P_2 purinoceptor-mediated effect on Ca^{2+} influx (Chen *et al.* 1995a) and on gonadotroph secretion (Chen *et al.* 1995a, Tomic *et al.* 1996) have been demonstrated. ATP is co-stored with neuropeptides in the secretory

granules of neurohypophyseal nerve endings in millimolar concentrations (Gratzl *et al.* 1980) and cytochemical localization of ectoATPase enzyme, terminating the action of extracellular ATP (cf. Zimmermann 1996), has also been shown in the neurohypophysis (Thirion *et al.* 1996), which permits the hypothesis that a similar autocrine/paracrine role of ATP also exists in the posterior lobe of the hypophysis. However, crucial evidence, i.e. stimulation-dependent ATP release and P_2 purinoceptor-mediated action on hormone secretion, is missing in this respect.

There are reports showing that ATP receptors are involved in the brainstem–pituitary pathway regulating vasopressin (AVP) secretion (Day *et al.* 1993, Buller *et al.* 1996) and our previous study showed that ATP is co-released with noradrenaline from nerve terminals of noradrenergic ascending bundle-innervating neurosecretory cells of the hypothalamus (Sperlágh *et al.* 1998b). In the present study we examined the possibility of whether ATP is released from the lower part of this pathway, downstream from the hypothalamus, i.e. from the posterior lobe

of the pituitary; its inactivation by sequential hydrolysis by ectoATPase and ecto5′-nucleotidase was also studied. In addition, the regulatory influence of endogenous ATP via P_2 purinoceptors was also demonstrated.

Materials and Methods

Male and female Wistar rats (Richter Gedeon, Budapest, Hungary) weighing 180–220 g were used. Animals were housed in groups of four or five and kept under constant indoor conditions (20 °C, 12 h light–12 h darkness cycles) for at least 1 week before the experiments. They had free access to standard commercial pellets and drinking water. On the day of experiment, rats were killed by decapitation and brains were quickly removed. The neural and intermediate lobes of the pituitary gland were dissected from the anterior lobe under a stereomicroscope and immersed in ice-cold Krebs' solution (containing (mM): NaCl 113, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25·0, glucose 11·5) bubbled with a mixture of 95% O_2 and 5% CO_2. The neural lobe was subsequently separated from intermediate lobe cells, as described earlier (Kárteszi *et al.* 1981) and four neurohypophyses were placed into one of four polypropylene 100 µl superfusion chambers (Vizi *et al.* 1985) and perfused continuously with Krebs' solution preheated to 37 °C and gassed with 95% O_2 and 5% CO_2. After a 70 min preperfusion period, perfusate samples were collected at 3 min intervals for the assay of ATP, AVP and oxytocin.

Electrical field stimulation was applied twice (S_1, S_2) through a pair of platinum ring electrodes at 30 min intervals by means of a Grass S88 stimulator (Quincy, MA, USA); square-wave pulses of 3 ms duration were delivered at 25 V, 10 Hz for 36 s (i.e. 360 shocks/stimulation period were applied). A similar stimulation paradigm has been used in previous studies to study stimulation–dependent release of ATP from different brain slice preparations (Cunha *et al.* 1996, Sperlágh *et al.* 1998*a,b*) in response to neural activity.

Assay of ATP and ADP

ATP released from the preparations was assayed using the luciferin–luciferase technique, as previously described (Sperlágh *et al.* 1995). Briefly, 100 µl aliquots of the samples were added to 40 µl of ATP assay mix (Sigma, St Louis, MO, USA) containing 0·33 mg firefly luciferase, 0·83 mg luciferin, 6 mg $MgSO_4$, 2 mg EDTA, 0·08 mg dithiothreitol, 5 mg BSA and 45 mg tricine, reconstituted in 5 ml sterile water and the luminescence was measured in a BioOrbit 1250 luminometer (Turku, Finland) for 15 s and analysed with LUMINO 1250 software (Turku, Finland). Before the experiments a standard calibration curve was prepared from different concentrations of ATP ranging from 10^{-9} to 10^{-12} M, and a high correlation

could be observed between light emission and ATP concentration ($r=0·997$). The actual ATP levels in the samples were calculated by a double log curve fitting program and expressed in pmol/g of tissue. The Krebs' solution with or without drugs was assayed for background ATP content. The increase in the release of ATP caused by electrical field stimulation (S_1, S_2) was calculated by subtracting the resting release measured during the pre-stimulation period from the release during the stimulation and after the stimulation period, and expressed in pmol/g. The effect of drugs on ATP release was expressed as the ratio of S_2 over S_1 in the presence and absence of the drug. Drugs were added to the perfusion fluid between S_1 and S_2, 18 min prior to S_2, and were kept in the solution throughout the experiment.

AVP and oxytocin assay

AVP and oxytocin levels in the perfusate samples were determined by RIA as described earlier (Laczi *et al.* 1986, Vecsernyés *et al.* 1994) and expressed in pmol/mg tissue.

ATP metabolism studies

Neurohypophyses were dissected from rats as described above, subdivided into three pieces and incubated in 3 ml Krebs' solution at 37 °C, bubbled with 95% O_2 and 5% CO_2, and 60 nmol ATP added to the 3 ml bath. Aliquots of 70 µl were taken after 2·5, 5, 10, 15, 20 and 30 min. The concentrations of AMP, ADP and ATP, after application of ATP, were measured by HPLC combined with ultraviolet light detection (HPLC-UV) according to the method described earlier (Sperlágh *et al.* 1995). The separation was carried out at room temperature with an analytical column (15 × 0·4 cm) and a guard column (2 × 0·4 cm) packed with Nucleosil C-18, particle size 3 µm (Bio Separation Technology, Budapest, Hungary). The mobile phase was 50 mM potassium phosphate with 1% (v/v) methanol at pH 5·5. The flow rate was 0·5 ml/min. Twenty microlitre aliquots of the samples were injected directly onto the column. The mobile phase was filtered through a 0·45 µm type HA Millipore filter (Millipore, Bedford, MA, USA) and degassed before use. Peaks corresponding to ATP, ADP, AMP and adenosine were monitored by UV detection at 250 nm with a Model C-R6 A integrator (Shimadzu, Tokyo, Japan). The nucleotides were quantified with the standard addition methods from the peak area in the chromatogram. A linear correlation between the peak area and the injected amount was observed for all the nucleotides. The actual concentrations of ATP, ADP and AMP were expressed as µM.

For the determination of the kinetic parameters of ectoATPase and ecto5′–nucleotidase, a linear regression for ATP or AMP concentrations as a function of time was calculated from the concentrations of the first five samples (0, 2·5, 5, 10 and 15) after three different initial

concentrations of ATP or AMP (20, 100 and 500 µM) and the slope was used as initial velocity (v_i). The v_i values were used for calculation of the parameters from the Lineweaver–Burk plot using linear line regression:

$$1/v_i = K_m/v_{max} \times [S] + 1/v_{max}$$

where v_i is the velocity measured when very little substrate has reacted, [S] is the concentration of the substrate, v_{max} (maximal velocity) is a point where the enzyme is saturated with the substrate and K_m (Michaelis constant) is the concentration that produces half-maximal velocity.

Materials

The following chemicals were used: ATP assay mix, ATP, ADP, AMP, tetrodotoxin (TTX) (all from Sigma) and suramin hexasodium (Bayer, Leverkeusen, Germany).

Statistics

The results were evaluated by one-way ANOVA followed by Dunnett's test (multiple comparisons), or Student's *t*-test or Welch test (pairwise comparisons). *P* values of less than 0·05 were considered statistically significant. All data were expressed as means ± S.E.M.

Results

Stimulation-dependent release of endogenous ATP, AVP and oxytocin from the isolated posterior lobe of the hypophysis

After a 70 min preperfusion period, the basal release of ATP was measured by the luciferin–luciferase assay (85·08 ± 7·18 pmol/g tissue in 3 min, $n=6$) and remained constant during the subsequent sample collection period (Fig. 1A). The basal release of AVP and oxytocin was 60·88 ± 21·82 ($n=6$) and 106·9 ± 31·02 pg/mg ($n=6$) respectively. Electrical field stimulation (10 Hz, 2·5 ms, 360 shocks) increased the release of ATP to the superfusate, yielding 234·9 ± 30·49 pmol/g evoked release ($n=6$). The amount of ATP released by the subsequent stimulation period was less, resulting in an S_2/S_1 ratio of 0·54 ± 0·06 ($n=6$). The release of AVP and oxytocin was also substantially increased in response to electrical stimulation (Fig. 1B and C); the evoked release of AVP and oxytocin was 2404 ± 313 and 6865 ± 917 pg/mg tissue ($n=6$) respectively. After 30 min, the second stimulation released less AVP and oxytocin, resulting in S_2/S_1 ratios of 0·69 ± 0·07 and 0·42 ± 0·09 ($n=8$) respectively.

Figure 1 shows that the onset and the fade of the stimulation-evoked outflows of ATP and posterior lobe hormones were not entirely identical. The response of ATP to stimulation was rapid, its peak appeared promptly in the sample collected during stimulation and faded in the next 3 min sample, while the outflows of AVP and oxytocin were longer-lasting, reaching their peaks 6 min after stimulation and declining in the next 18 min. Furthermore, inhibition of the voltage-dependent Na^+ channels by TTX (1 µM) almost completely abolished the evoked release of AVP and oxytocin, while the release of ATP remained unaffected (Fig. 2), showing that the hormone release, but not ATP release in response to stimulation, is correlated with action potential propagation along the neuronal membrane.

Effect of P_2 receptor activation on hormone secretion

The ability of endogenous ATP to affect the secretion of AVP and oxytocin in the neural lobe of the hypophysis was assessed by the use of suramin, a specific antagonist of P_2 type purinoceptors (Fig. 3). Suramin (300 µM) applied to the superfusion fluid 15 min prior to the second stimulation significantly increased the amount of oxytocin and AVP released by stimulation, suggesting that the tonic action of endogenous ATP decreases the release of AVP and oxytocin, an effect mediated by P_2 purinoceptors. ATP (1 mM), by itself, diminished the release of AVP but not that of oxytocin (Fig. 3), and its effect was reversed by suramin (300 µM); the S_2/S_1 ratios were 0·36 ± 0·04 and 1·43 ± 0·31 in the presence of ATP and ATP plus suramin respectively ($n=5$, $P<0·05$).

ATP metabolism studies

The hydrolysis rates of 60 nmol ATP and AMP added to 3 ml incubation medium were also measured in the preparations by the HPLC-UV technique, by assaying ATP, ADP and AMP concentrations in the bathing fluid after drug administration (Fig. 4). ATP was readily decomposed by the enzymatic action of ectoATPase; conversely, the amount of ADP and AMP was increased (Fig. 4A). Similarly, when 60 nmol AMP were added to the tissues, its amount declined, and adenosine appeared in the extracellular fluid (Fig. 4B), showing the activity of ecto5′-nucleotidase enzyme. The kinetic parameters of the ectoATPase and ecto5′-nucleotidase were determined by initial rate measurements (Sperlágh *et al.* 1995); the K_m and v_{max} values of ectoATPase were 267 ± 52·68 µM and 6·70 ± 1·08 nmol/min per preparation ($n=3$) respectively, and 334 ± 165 µM and 2·54 ± 0·24 nmol/min per preparation ($n=3$) respectively for ecto5′-nucleotidase.

Discussion

Neurohypophyseal regulation is conveyed on different levels; oxytocin and AVP secretion from the terminals of magnocellular neurons of the supraoptic nucleus is controlled by factors circulating in the blood and neuronal factors acting on the cell body in the hypothalamus. In
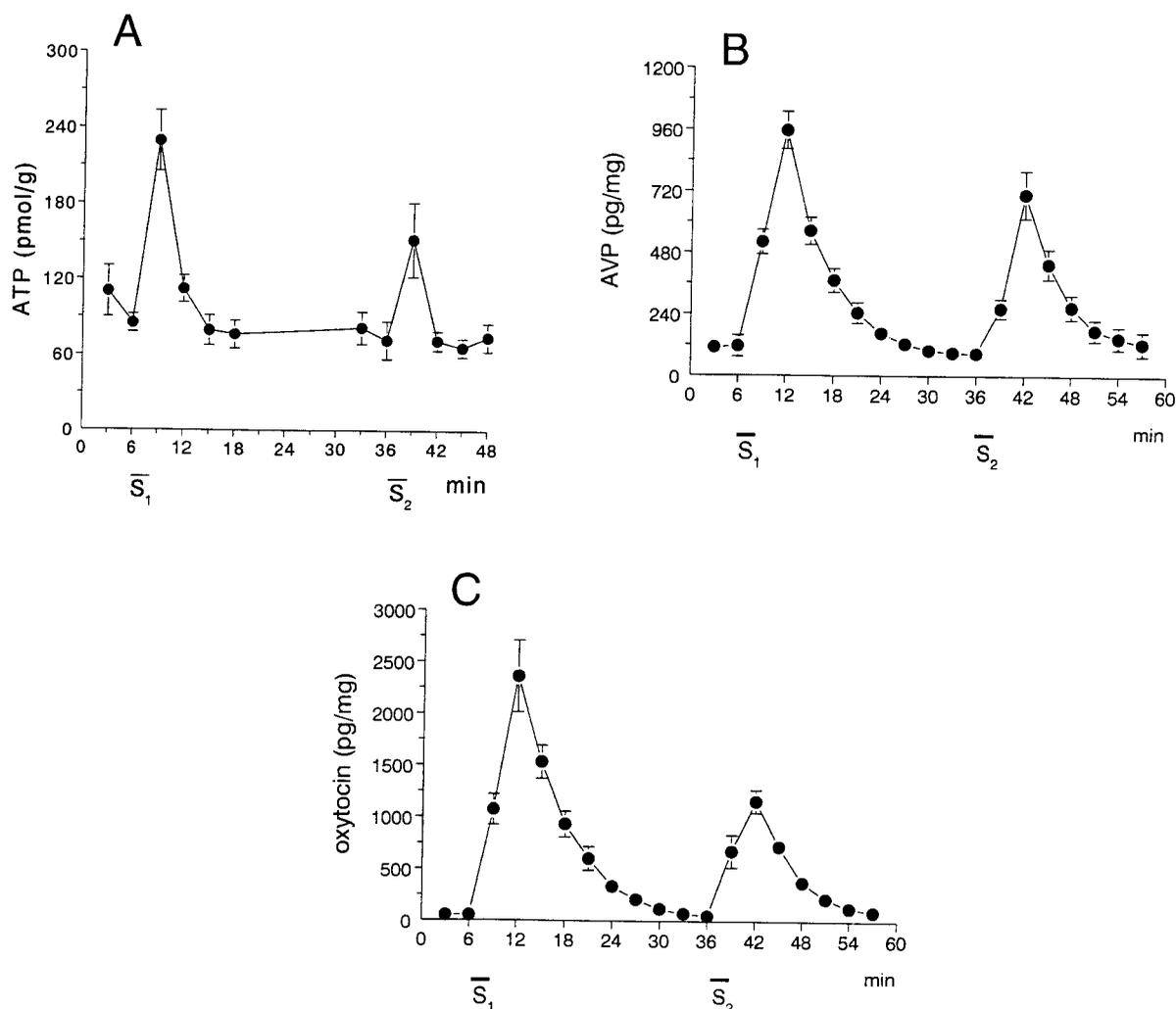
Figure 1 Electrical field stimulation-induced release of ATP (A), AVP (B) and oxytocin (C) from the isolated posterior pituitary preparation. Tissues were superfused with Krebs' solution for 70 min, stimulated electrically twice (S_1, S_2, 10 Hz, 3 ms, 360 shocks) and perfusate samples collected. ATP levels in the samples were measured by the luciferin–luciferase assay, AVP and oxytocin by RIA. The release of ATP was expressed in pmol/g, that of AVP and oxytocin in pg/mg, as a function of time. Data are means ± S.E.M. of eight identical experiments.

addition, regulation at the terminal level by autocrine/paracrine mediators via a non–synaptic mechanism (Vizi & Lábos 1991) also appears to be important.

The question addressed in this study was whether purinergic signalling mechanisms might operate at the terminal level of this regulatory cascade, i.e. in the isolated posterior lobe of the hypophysis. If a compound acts as an intercellular signal the following conditions have to be fulfilled: (i) it has to be released into the extracellular milieu in response to a physiological activation signal; (ii) it has to be removed from the extracellular space by an inactivation system; and (iii) it has to affect the function of the target cells via specific receptor-mediated mechanisms.

In our experiments, the release of endogenous ATP, measured by the luciferin–luciferase assay, and of AVP and oxytocin were simultaneously detected from the same preparations. Electrical field stimulation at 10 Hz, which is close to the physiological firing patterns of AVP neurons (average frequency of 12–13 Hz, Bondy *et al.* 1988), elicited a rapid release of ATP, reaching a more than twofold increase above the background ATP level. AVP and oxytocin were also released in a remarkable amount in response to stimulation; nevertheless, the time courses of the release of ATP and hormone secretion were not identical. The peak of the ATP release appeared promptly after the stimulation period, and the response returned to
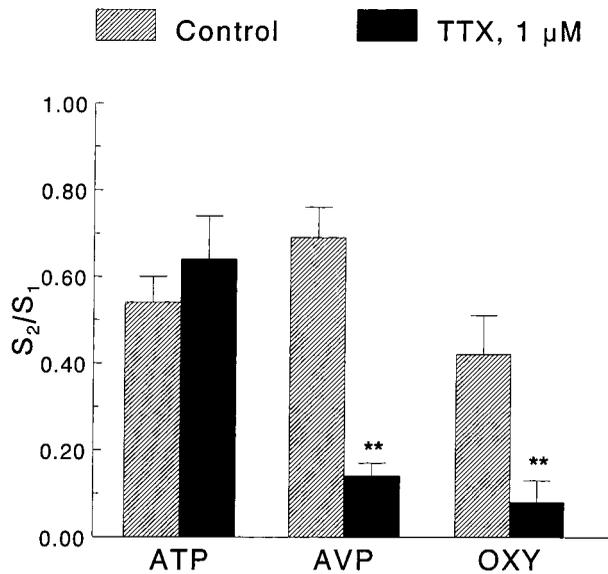
Figure 2 Effect of TTX (1 µM) on the evoked release of ATP, AVP and oxytocin. TTX was administered into the perfusion fluid 18 min prior to the second stimulation period (S_2). Its effect on the stimulation-evoked release of ATP, AVP and oxytocin (OXY) was expressed as S_2/S_1 ratios. Asterisks represent significant differences between control groups and TTX-treated groups (**$P<0.01$, $n=5$).

the baseline in the next 3 min period. Conversely, the secretions of oxytocin and AVP were delayed, reaching their peak 6 min after stimulation and lasted for 18 min. Evidently different ATP and hormone release profiles may be explained by the fact that the posterior lobe preparation is 0·5–1 mm thick and different compounds will take different times to diffuse from within the tissue to the perfusion fluid. As indicated by ATP breakdown experiments (see Fig. 4), ATP is rapidly metabolized in the extracellular space by ectonucleotidases, and a part of the released ATP is probably hydrolysed before diffusion to the perfusate, resulting in a short-lasting response. Presumably, AVP and oxytocin have longer extracellular half-lives and their outflows were more prolonged than the outflow of ATP. The question arises: what is the cellular and subcellular origin of released ATP, and is it identical with that of posterior pituitary hormones?

While AVP and oxytocin are derived from the neurosecretory nerve terminals of the neurohypophysis, there are three potential source of extracellular ATP: (i) afferent nerve terminals terminating in the neurohypophysis; (ii) pituicytes, specified glial cells of the posterior lobe; and (iii) neurosecretory nerve endings. As ATP is present in the neurosecretory granules (Gratzl *et al.* 1980), the origin of the released ATP should, at least partly, be a common or separate pool of the granules. However, abolition of axon
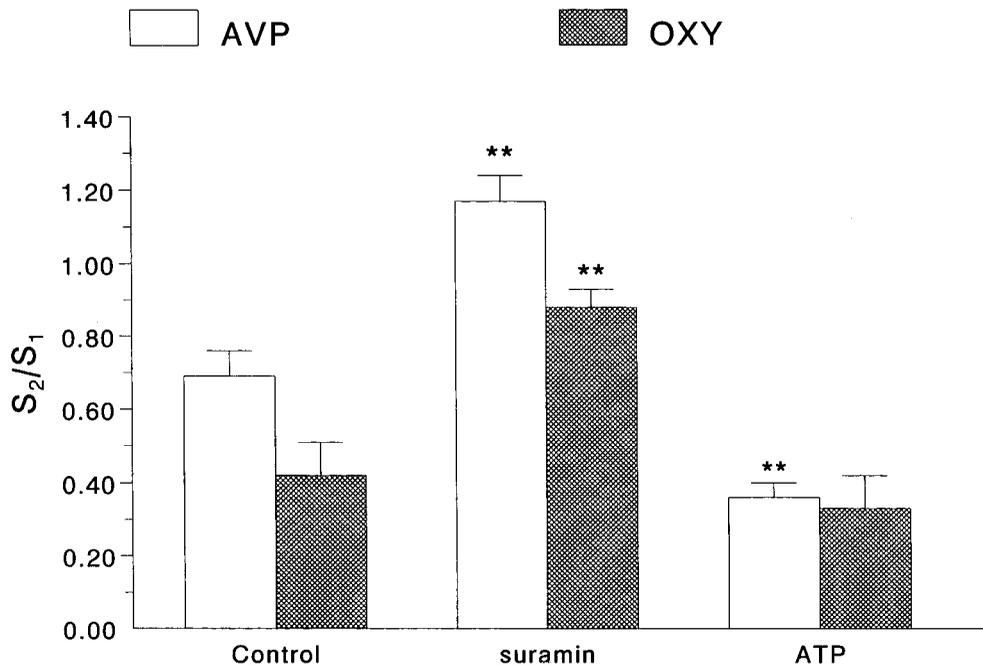

Figure 3 Effect of suramin and ATP on the evoked release of AVP and oxytocin (OXY). Suramin and ATP were administered into the perfusion fluid 18 min prior to the second stimulation period (S_2). Their effect on the stimulation-evoked release of AVP and oxytocin was expressed as S_2/S_1 ratios. Asterisks represent significant differences between control groups and drug-treated groups (**$P<0.01$, $n=4$–6).
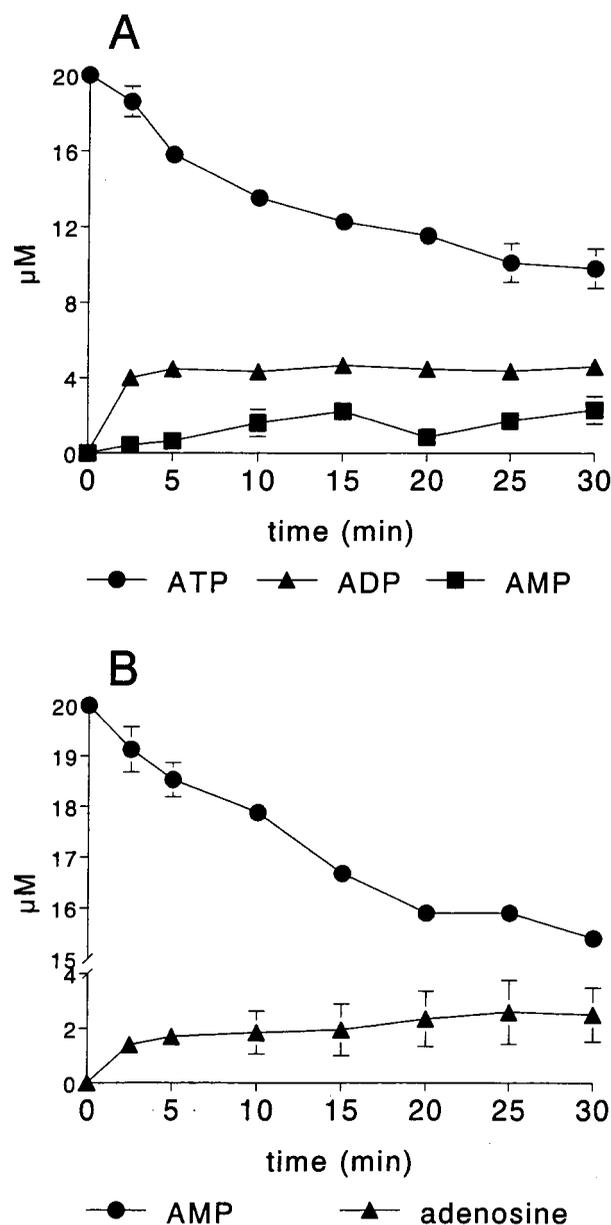
Figure 4 EctoATPase (A) and ecto5′-nucleotidase (B) activity in the isolated posterior pituitary preparation. Preparations were incubated in 3 ml Krebs' solution in the presence of 60 nmol ATP. Aliquots (70 µl) were taken 2·5, 5, 10, 15, 20, 25 and 30 min after the addition of ATP. The amounts of nucleotides and adenosine in the aliquots were determined by the HPLC-UV method and expressed as µM. Data are the means ± S.E.M. of three identical experiments.

potential propagation by TTX strongly reduced hormone secretion, while the release of ATP remained unaffected, which indicates that the majority of ATP and posterior lobe hormones represented in the outflow have separate origins. The earlier and sharper peak of the release of ATP

also suggests that the source of ATP is not identical to that of AVP and oxytocin. However, one also has to consider the fast extracellular hydrolysis of ATP (see Fig. 4), which prevents the capture of the total amount of released ATP. As for the TTX-resistant part of the release, its cellular source might be either the afferent nerve terminals or the pituicytes. In the lack of axonal conduction, i.e. in the presence of TTX, membranes can be stimulated directly by the applied electrical current. Depolarization-induced release of transmitters by electrical stimulation has been described from glial cells (Dennis & Miledi 1974), and a similar, stimulation-dependent, TTX-resistant release of endogenous ATP was observed in the capsula glomerulosa preparation of the adrenal gland where steroid hormone secretion is regulated by P_2 receptors (Jurányi *et al.* 1997). Boersma *et al.* (1993) observed synaptoid contacts between pituicytes and oxytocin- and AVP-containing axon terminals in the rat neural lobe, which provides an ultrastructural correlate for a local release of ATP from pituicytes in response to nerve terminal depolarization. Nevertheless, further studies are necessary to identify the exact source of released ATP. Another intriguing question is how the release of ATP is altered during other physiological stimuli regulating AVP and oxytocin secretion, such as osmotic stimulation, dehydration or stress.

The question arises: what is the functional consequence of local ATP release in the neurohypophysis? It is well known that oxytocin and AVP release are subject to modulation by a number of different receptors, including dopamine (Vizi & Volbekas 1980), κ-opioid (Leng *et al.* 1994), neuropeptide Y (Larsen *et al.* 1994) and γ-aminobutyric acid-A (GABA-A) (Magnusson & Meyerson 1993) receptors, and by nitric oxide (Lutz-Bucher & Koch 1994, Pow 1994) at the pituitary level. The P_2 purinoceptor family is a growing receptor family, which is divided into the ionotropic (P_{2x}) and metabotropic (P_{2y}) receptor subfamilies; seven members of each subfamily have been molecularly identified so far (Buell *et al.* 1996). Since P_{2x} receptors are present in the pituitary (Brake *et al.* 1994, Chen *et al.* 1995*b*, North 1995) and Ca^{2+} currents in isolated neurohypophyseal nerve terminals have been shown to be modulated by ATP (Wang & Lemos 1993, Troadec *et al.* 1998), it appears a reasonable idea that hormone secretion is under the regulatory influence of ATP, via P_2 receptors. In this study, suramin, the specific P_2 receptor antagonist, was used to test this possibility, i.e. to displace the action of endogenous ligand on P_2 receptors. Suramin, which possesses extended selectivity and acts on a wide array of both P_{2x} and P_{2y} subtypes (Burnstock 1997), increased the release of both oxytocin and AVP at a concentration at which it exhibits a P_2 receptor antagonist property (300 µM, Dunn & Blakeley 1988), indicating that hormone secretion is under the inhibitory influence of ATP released *in situ* by nerve stimulation. However, recent studies indicated that suramin does not block exclusively P_2 purinoceptors; it

also inhibits glutamate and GABA receptor-mediated currents (Nakazawa *et al.* 1995), as well as the coupling of receptors to G proteins (Beindl *et al.* 1996). Since the posterior lobe receives a well-defined GABAergic inner-vation from central nuclei (Oertel *et al.* 1982, Vincent *et al.* 1982, Buijs *et al.* 1987) and AVP release in the pituitary is modulated by GABA-A receptors (Anderson & Mitchell 1986, Sladek & Armstrong 1987, Magnuson & Meyerson 1993), augmentation of oxytocin and AVP release by suramin could be explained by the removal of GABAergic regulatory influence. Glutamate immuno-reactivity (Meeker *et al.* 1991) and metabotropic glutamate receptor subtypes (Kiyama *et al.* 1993) are also present in neurosecretory nerve terminals, as well as in pituicytes, and therefore glutamatergic modulation of hormone secre-tion has also to be considered. However, the finding that ATP reduced the release of AVP, and suramin prevented the inhibitory effect of ATP on hormone secretion, favours the assumption that the action of suramin is related to P_2 purinoceptors and the efflux of at least AVP is regulated by endogenous ATP. Although a high concentration of ATP (1 mM) was needed to obtain a significant effect, taking into account the rapid hydrolysis of ATP in the extracel-lular space, the concentration of ATP at the receptor site was probably much lower. Furthermore, the probability that ATP, added exogenously, competes for receptor sites with endogenously released ATP (see Figs 1A and 3), offers a further explanation for the relatively weak effect of exogenous ATP on hormone secretion.

In the third part of the study, the extracellular break-down of ATP was examined using the HPLC-UV tech-nique. When 60 nmol ATP was added to the tissues, it was readily hydrolysed to ADP and AMP, showing the activity of ectoATPase, an enzyme which is widely but not unevenly distributed in neural and non-neuronal tissues (Zimmermann 1996). These observations are consistent with the enzyme cytochemical findings of Thirion *et al.* (1995), who found that ectoATPase precipitates are abun-dantly localized around neurosecretory nerve terminals and pituicytes in the neurohypophysis. The K_m value of ectoATPase was in the high micromolar range, which indicates that its activity is high enough to metabolize the ATP amounts released upon depolarization. Therefore, in view of the fast extracellular hydrolysis of released ATP, the biophase concentration of ATP at release sites is probably higher than we detected in the release exper-iments. Furthermore, in our experiments, the activity of ecto5′-nucleotidase enzyme, which is responsible for the hydrolysis of AMP to adenosine, was also shown. These findings indicate that ATP is sequentially metabolized in the extracellular matrix to ADP, by ectoATPase, and to AMP and adenosine by the ecto5′-nucleotidase, and the end-product of this inactivation process is adenosine. Since adenosine is a well-known neuromodulator, which is inactive on ATP receptors, but is active on adenosine receptors (cf. Windscheif 1996), our findings invite further

investigation on possible adenosine receptor-mediated actions in the posterior pituitary. Otherwise, adenosine, accumulating in the extracellular fluid, is able to pass through the membrane by nucleoside transporters and is available for the resynthesis of ATP inside the cell.

In summary, our results clearly demonstrate that en-dogenous ATP is released stimulation–dependently and metabolized extracellularly in the rat isolated posterior pituitary. In addition, AVP secretion has been shown to be modulated by endogenous and exogenous ATP via P_2 receptors, supporting the idea that ATP acts as an inter-cellular messenger in the neural lobe of the hypophysis.

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