Proadrenomedullin N-terminal 20 peptide (PAMP) stimulates aldosterone secretion by the intact rat adrenal glomerulosa by a cAMP-dependent mechanism

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Received 26 May 1998; revised version received 1 July 1998

Abstract Preproadrenomedullin, the precursor of proadrenomedullin N-terminal 20 peptide (PAMP), is produced by rat zona glomerulosa cells. The actions of PAMP on the rat adrenal have been investigated. PAMP was found to stimulate aldosterone secretion and cAMP release by intact capsules, in a dose-dependent manner, but had only a minor effect on dispersed cells. The effects of PAMP on aldosterone secretion were inhibited by HA1004, an inhibitor of protein kinase A. The difference between tissue preparations does not appear to be due to the actions of PAMP on local release of catecholamines as PAMP inhibited the release of catecholamines from rat capsular preparations. These data suggest that PAMP is a novel zona glomerulosa stimulant in intact capsular tissue, acting through cAMP.

Key words: Proadrenomedullin N-terminal 20 peptide; Aldosterone; Zona glomerulosa; cAMP

1. Introduction

Proadrenomedullin N-terminal 20 peptide (PAMP) is a recently identified cleavage product of preproadrenomedullin, which is expressed in a wide range of different human tissues [1]. We have recently demonstrated that the gene encoding preproadrenomedullin is expressed in the rat adrenal zona glomerulosa [2]. Specific binding sites for PAMP have been identified in many different tissues in the rat, including the adrenal glands [3]. More recently specific PAMP receptors have been identified in the rat adrenal zona glomerulosa, and there is evidence that activation of the PAMP receptor leads to an increase in cAMP production in this tissue [4]. This is consistent with findings in other tissues, such as the cat mesenteric vascular bed, in which the effect of PAMP is mediated by cAMP [5]. In the light of these observations it may be hypothesised that PAMP is a stimulus to aldosterone secretion, in common with other agents which elevate cAMP levels in the zona glomerulosa. However, the one group which has, to date, studied the actions of PAMP on the rat zona glomerulosa suggests that this peptide inhibits aldosterone secretion [6,7].

The present study was designed to further investigate the actions of PAMP on cAMP and aldosterone secretion by the rat adrenal zona glomerulosa. The intact capsular preparation was chosen because this tissue preparation retains some of the architecture of the gland, and is likely to be more relevant to the in vivo situation than a dispersed cell preparation. In particular, previous studies from our laboratory and others have suggested that, in the rat adrenal zona glomerulosa, peptide-stimulated local release of catecholamines from islets of chromaffin cells located within the zona glomerulosa may have a role in intraglandular signal transduction and may also account for differences in responses seen between different tissue preparations [8]. This possibility was also addressed by the present studies by investigating the effects of PAMP on capsular catecholamine release.

2. Materials and methods

All chemicals were obtained from Sigma Chemical Co. (Poole, Dorset, UK) or BDH (Poole, Dorset, UK), with the following exceptions: proadrenomedullin N-terminal 20-peptide (PAMP), [^{125}I]PAMP and rat adrenomedullin were obtained from Phoenix Pharmaceuticals (Mountain View, CA, USA), and HA1004 was bought from Semat Laboratories (St Albans, Herts., UK). ACTH1-24 (Synacthen) was obtained from Ciba-Geigy (Horsman, Surrey, UK).

Female Wistar rats (body weight 250–350 g) were obtained from the colony at Queen Mary and Westfield College, and maintained on a normal rat diet with food and tap water available ad libitum. Animals were rapidly killed by mechanical stunning followed by cervical dislocation. Adrenal glands were excised and glomerulosa tissue separated from inner zones and medulla by gentle compression between glass plates. Adrenal capsules were prepared and incubated in Krebs bicarbonate Ringer as previously described [9] in the presence or absence of stimulants. At the end of the incubation period tissues were removed from the medium, which was then stored frozen at −20°C until assayed for aldosterone and cAMP.

Zona glomerulosa and zona fasciculata/reticularis cell suspensions were prepared by collagenase digestion and mechanical dispersal as previously described [10]. Zona glomerulosa cells and inner zone (zona fasciculata/reticularis) cells were separately incubated in Krebs bicarbonate Ringer containing 2 mg glucose/ml and 2 mg bovine serum albumin (fraction V)/ml. Cells were incubated in Eppendorf tubes at a density of 10 000/ml in an incubation volume of 1 ml, under an atmosphere of 95% O_2/5% CO_2 at 37°C for 60 min. Peptide additives were dissolved in incubation medium and added as required. At the end of the incubation cells were pelleted by centrifugation, medium removed and stored at −20°C.

Aliquots of incubation medium were assayed for aldosterone (zona glomerulosa cells and capsules) and corticosterone (inner zone cells) by radioimmunoassay as previously described [10]. cAMP was measured by competitive protein binding assay as previously described [10]. All experiments were carried out in duplicate and repeated at least three times.

The role of cAMP-dependent mechanisms in the action of PAMP on the zona glomerulosa was investigated using HA1004 (Semat Technical, St Albans, Herts., UK), a selective inhibitor of protein kinase A at the concentration used (1 μmol/l) [11]. Adrenal capsules were in-

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PII: S0014-5793(98)00828-X

cubated as described above, with 100 nmol/l PAMP, in the presence and absence of HA1004 (1 μmol/l).

Catecholamine release was measured by assaying the total adrenaline and noradrenaline content of incubation medium from intact capsules using a fluorescent method as previously described [2].

Dose response data were analysed using analysis of variance (ANOVA). For other data the Student's *t*-test was used.

3. Results

PAMP caused a dose-dependent increase in aldosterone secretion by intact rat adrenal capsular tissue incubated in vitro (Fig. 1). The minimum concentration of PAMP required for significant stimulation of aldosterone secretion was found to be 10 nmol/l. The maximal effect of PAMP was seen at a concentration of 1 μmol/l (the highest concentration tested). At this concentration PAMP caused a four-fold increase in aldosterone secretion compared with basal levels. PAMP also caused a significant increase in cAMP release from rat adrenal capsular tissue (Fig. 1). The minimum concentration of PAMP required for this action was 10 nmol/l. The maximal increment in cAMP was a twofold increase over basal. The effects of PAMP on aldosterone secretion by intact capsular tissue were significantly attenuated in the presence of 1 μmol/l HA1004, a protein kinase A inhibitor. This inhibitor also significantly attenuated the aldosterone response to ACTH stimulation (Fig. 2).

In dispersed zona glomerulosa cells, however, PAMP only had a very small effect on basal aldosterone secretion (Fig. 3), although a highly significant dose-dependent increase in cAMP release was observed (Fig. 3). At a concentration of 1 μmol/l PAMP caused a 50% increase in aldosterone secretion over basal levels, while there was a fourfold increase in cAMP secretion. PAMP did not affect corticosterone secretion by zona fasciculata/reticularis cells at any concentration used (data not shown).

PAMP at a concentration of 100 nmol/l was found to cause a significant decrease in the release of catecholamines from adrenal capsular tissue (Fig. 4).

4. Discussion

The data obtained in the present study suggest that PAMP acts on the intact rat adrenal zona glomerulosa to stimulate aldosterone secretion. These data, showing an increase in cAMP production in response to PAMP, together with inhibition of the response in the presence of a specific PKA antagonist suggest that the adrenocortical response to PAMP is mediated by cAMP as second messenger. This is in accordance with our previous studies demonstrating the presence of specific PAMP receptors linked to adenylyl cyclase in the rat zona glomerulosa [4]. It is not clear, however, why dispersed cells do not respond in the same way to PAMP stimulation. The apparent paradox presented by the observation of a fourfold increase in cAMP release with less than a twofold change in aldosterone secretion may be explained if PAMP were acting on one of the other cell types known to be present in this preparation: vascular endothelial cells for example. The cAMP released from such cells would not be available to the zona glomerulosa cells owing to the poor ability of cAMP to penetrate cells.

Previous studies of the effects of PAMP on aldosterone secretion from Nussdorfer's group also found that PAMP had no effect on basal aldosterone secretion by dispersed zona glomerulosa cells, although they reported an inhibitory

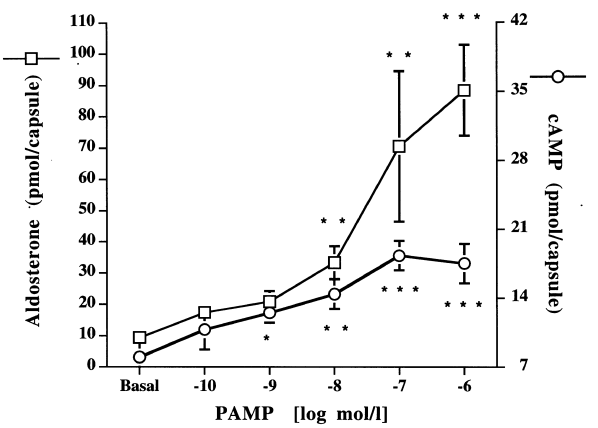

Fig. 1. Effects of PAMP on aldosterone secretion (square symbols) and cAMP release (round symbols) by intact rat adrenal capsular tissue incubated in vitro. Data are means ± S.E.M., $n=6$. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to basal values (ANOVA).

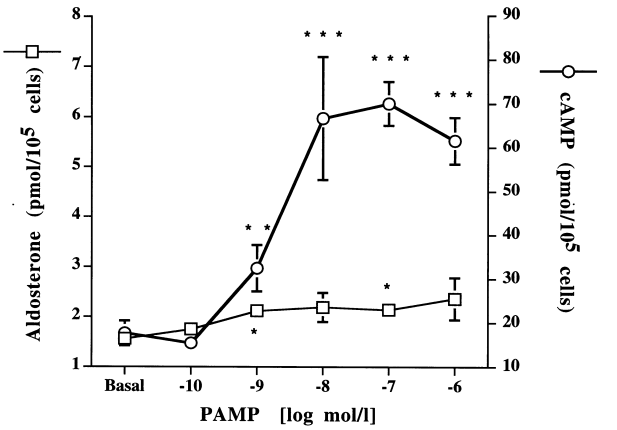

Fig. 2. Effects of PAMP on aldosterone secretion (square symbols) and cAMP release (round symbols) by collagenase-dispersed rat adrenal zona glomerulosa cells incubated in vitro. Data are means ± S.E.M., $n=6$. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to basal values (ANOVA).

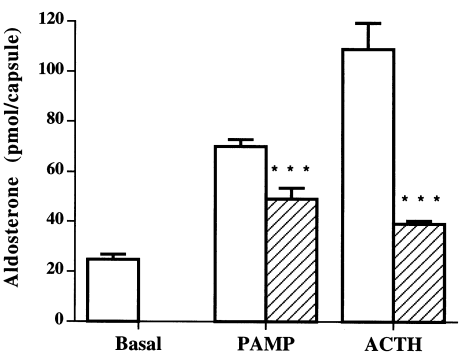

Fig. 3. Effects of the protein kinase A inhibitor HA1004 (1 μmol/l) on the aldosterone response to PAMP (100 nmol/l) and ACTH (1 nmol/l) in intact capsular tissue. Open bars show response in the absence of HA1004, hatched bars show response in the presence of HA1004. Data are means ± S.E.M., $n=6$. $^{***}P<0.001$ compared to response in the absence of HA1004 (Student's *t*-test).

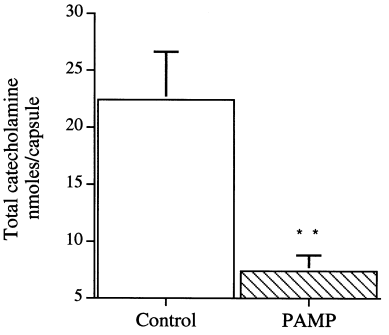

Fig. 4. Effects of PAMP (100 nmol/l) on the release of catecholamines from rat adrenal capsular tissue incubated in vitro. Data are means ± S.E.M., $n = 6$. $^{**}P < 0.01$ compared to control (basal) levels (ANOVA).

effect of PAMP on angiotensin II-stimulated aldosterone secretion [6,7]. The discrepancy between these two tissue preparations is similar to the effects of vasoactive intestinal polypeptide (VIP), which has no effect on basal aldosterone secretion by dispersed cells, but stimulates intact tissue [8]. Adrenomedullin has also been reported to exert opposite effects on dispersed cells and intact capsular tissue, like PAMP, causing stimulation of aldosterone in capsules [9] or other intact adrenal preparations [12] while inhibiting aldosterone secretion by cells [6,7,13,14], although it should be noted that the actions of adrenomedullin on dispersed cells also appear to depend on the receptor subtype which predominates in the population of animals used [2]. It is entirely possible that inherent differences in the rat populations used for these experiments may account for the differences seen. It appears to be a possibility that part of the effect of PAMP is mediated by the adrenomedullin receptor, as PAMP and adrenomedullin share a common binding site [4]. This is a major difference between the populations of rats used by our group, which expresses a specific adrenomedullin receptor [2], and the animals used by Nussdorfer's group, in which all the actions of adrenomedullin are mediated by the CGRP receptor [13].

There remains, however, the question as to why PAMP is a potent stimulus to aldosterone secretion in intact capsular tissue but not in dispersed cells. An explanation which has been offered for the differing effects of a peptide on intact tissue and dispersed cells is the existence of intraglandular signal transduction mechanisms. These include the local release of catecholamines from either nerves within the adrenal capsule/zona glomerulosa or from islets of chromaffin cells in the capsular region [8,15]. The possibility that PAMP may act in this manner was investigated by measuring catecholamine release by intact capsular tissue in response to PAMP. As PAMP was shown to cause a decrease in the release of catecholamines it is most unlikely that this mechanism may explain the difference in the response of the tissue preparations. The effect of PAMP on catecholamine secretion is consistent with a previous report of PAMP inhibition of catecholamine secretion by cultured bovine chromaffin cells [16].

In conclusion, it appears that PAMP is stimulatory to aldosterone secretion in intact tissue preparations, and this effect is mediated by cAMP. It is not clear why PAMP does not stimulate dispersed cell preparations, but this discrepancy may suggest the existence of as yet uncharacterised intraglandular signal transduction mechanisms.

Acknowledgements: We are grateful to the Royal Society for supporting these studies.

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