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Stability of the vasopressin V_2 receptor–adenylyl cyclase system in rat kidney

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Abstract

In the Brattleboro rat with diabetes insipidus vasopressin V_2 receptor mRNA and the mRNA of various adenylyl cyclase (AC) isoforms are moderately reduced compared with those of normal rats. In the present study renal vasopressin V_2 receptor mRNA was modestly higher (by 34%), as was expression of AC 5, 6 and 9 mRNAs (up to 22% greater), in BDI rats treated with the vasopressin V_2 receptor agonist desamino-[Arg^8] vasopressin than in untreated controls. AC 4 mRNA was decreased by 17% following desamino-[Arg^8] vasopressin treatment. While the stimulatory G$s\alpha$ mRNA was little affected by the desamino-[Arg^8] vasopressin treatment, two of the inhibitory G proteins were raised (Gαi-2 by 54% and Gαi-3 by 57%). Treatment of Sprague–Dawley rats with a specific vasopressin V_2 receptor antagonist (SR 121463A) was not associated with any marked changes in mRNA expression. These results indicate that the vasopressin V_2 receptor adenylyl cyclase system mediating the antidiuretic response to vasopressin is relatively stable. The Gi proteins may be involved in the stabilizing mechanism. © 1998 Elsevier Science B.V.

Keywords: Vasopressin; Vasopressin V_2 receptor; Adenylyl cyclase; G protein; Brattleboro rat

1. Introduction

Various endocrine systems are regulated not only by negative (or in rare instances positive) feedback loops acting back on the site of production of a hormone but also on the target tissue itself, for example by regulation of receptor numbers (either by up- or down-regulation) and/or by intracellular self-regulating pathways (e.g. on second messengers). One typical instance of receptor regulation is the well-described relationship between changes in catecholamine concentrations and their b-receptors. High circulating concentrations of adrenaline or other β-adrenoceptors agonists are associated with marked down-regulation of their receptors (Mukherjee et al., 1976) while chronic administration of β-adrenoceptor blocking drugs is associated with a large increase (up-regulation) in receptor numbers (Glaubiger and Lefkowitz, 1977), which can result in increased sensitivity of tissues to catecholamines (Aarons et al., 1980). Rapid inactivation of receptors (desensitization) is at least partly brought about by β-adrenoceptor kinases which, together with their co-factors the arrestins, uncouple the ligand-activated receptors from their G proteins. Longer-term desensitization involves the down-regulation of receptors, for which process possible mechanisms include either their enhanced removal (e.g. by increased degradation) or decreased synthesis (for recent review, see Bohm et al., 1997).

Vasopressin is another hormone which binds to membrane receptors, of which one type, the renal vasopressin V_2 receptor, is coupled to G proteins and is associated with adenylyl cyclase (AC) activation. The generation of cAMP in collecting duct cells is associated with protein kinase A phosphorylation. Ultimately, the antidiuretic action of the hormone is exerted by the insertion of aquaporin 2 protein water channels in apical membranes (for review see King and Agre, 1996). Desensitization of these receptors in various vasopressin-stimulated renal cell lines such as LLC-PK (pig kidney) (Lester et al., 1985) and Madin–Darby canine (Aiyar et al., 1990) cells has been reported, and a similar conclusion was reached in a study of vasopressin V_2 receptor-transfected cells of a mouse fibroblast

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cell line (Birnbaumer et al., 1992). A more physiological study also indicates that when circulating vasopressin concentrations are raised (13-fold) as a consequence of 24-h water restriction in rats, vasopressin V_2 receptor mRNA is decreased by approximately 33% (Terada et al., 1993). These various findings suggest that in the absence of circulating vasopressin the reverse situation should occur, namely that there would be vasopressin V_2 receptor up-regulation. However, a paradoxical reduction in renal vasopressin binding has been reported in the Brattleboro rat with hereditary diabetes insipidus (BDI), which lacks circulating vasopressin (Ravid et al., 1985) and has reduced adenylyl cyclase activity in response to vasopressin (Dousa et al., 1975; Rajerison et al., 1977). Recently, these paradoxical findings have been reinforced by observations that in BDI rats, unlike normal rats of the parent Long Evans (LE) strain, (a) vasopressin V_2 receptor mRNA is reduced, (b) there is a parallel reduction in total basal and vasopressin-stimulated adenylyl cyclase activity, and (c) there is reduced expression of the four main AC mRNA isoforms expressed in the kidney (Shen et al., 1997). While this vasopressin-deficient animal has a reduced renal expression of aquaporin 2 water channels (DiGiovanni et al., 1994), hormone replacement is associated with an increased insertion of these channels into the apical membranes of the collecting duct target cells (Yamamoto et al., 1995).

Thus, there is evidence of vasopressin V_2 receptor down-regulation under both vasopressin hypostimulated and hyperstimulated conditions, with both desensitization states being relatively moderate. However, there is no information about vasopressin V_2 receptor-G protein-adenylyl cyclase regulation in response to vasopressin V_2 receptor agonist or antagonist treatment. The present study was therefore designed to examine the effects of treatment with a specific vasopressin V_2 receptor agonist (desamino-[Arg^8] vasopressin) of BDI rats, and of treatment with an antagonist (SR 121463A, Serradeil-Le Gal et al., 1996) of normal rats in order to determine how the intracellular regulatory processes are influenced by ligand–receptor binding.

2. Methods

2.1. Drug treatments

2.1.1. Vasopressin V_2 receptor agonist-treated Brattleboro rat studies

Male adult Brattleboro rats with diabetes insipidus (BDI, $n = 8$) from Charing Cross and Westminster Medical School, UK, were housed in metabolic cages for two periods of 5 days each, separated by a 2 day gap, the last days of each period being used for the measurement of daily variables including fluid intake and urine volumes. During the second period four of the rats, chosen at random, received the vasopressin V_2 receptor agonist (desamino-[Arg^8] vasopressin (Ferring, Sweden), the other rats being maintained on tap water. The desamino-[Arg^8] vasopressin was added to the drinking water at a concentration of 2 mg/l according to a protocol used previously (Laycock, 1994), the fluid being replaced daily. All rats were given standard powdered diet and fluid ad libitum. On the fifth day of the treatment period, all rats were killed by decapitation and their kidneys were removed, decapsulated and frozen in liquid N_2 until required.

2.1.2. Vasopressin V_2 receptor antagonist-treated Sprague–Dawley rat studies

Adult male Sprague–Dawley rats obtained from Janvier, France, were divided into two groups, the rats of one group ($n = 8$) receiving treatment with the vasopressin V_2 receptor antagonist SR121463A (SR, Sanofi Recherche, France) administered as a twice-daily dose of 0.3 mg/kg BWt i.p., for 8 days. The rats of the other group ($n = 8$) received i.p. injections of drug vehicle (150 mM NaCl) and were used as controls, all animals being maintained on a standard diet and tap water ad libitum. At the end of the eighth day, urine samples were obtained from all rats for Na^+ and K^+ concentration determinations. Finally, all rats were killed by decapitation and their kidneys were removed, decapsulated and frozen in liquid N_2 until required.

2.2. Adenylyl cyclase activity

The procedure used for determining adenylyl cyclase activity has been described fully elsewhere (Shen et al., 1997). Briefly, frozen kidneys were weighed and placed in 10 vol. of an ice-cold buffer containing 50 mM Tris–HCl (pH 7.6), 2 mM EDTA (pH 7.6), 1 mM dithiothreitol and 0.1 mM phenylmethylsulfonyl fluoride and homogenized. The homogenate was centrifuged at $150 \times g$ at 4°C, the pellet was discarded and the supernatant was recentrifuged at $17{,}000 \times g$ at 4°C. This time the supernatant was discarded, and the pellet was resuspended in the same buffer and recentrifuged, this process being repeated three times. The final pellet of purified cell membranes was resuspended in an appropriate volume of the same buffer and stored at −80°C until required.

Frozen membrane preparations were then thawed and centrifuged at $17{,}000 \times g$ for 30 min at 4°C, and the pellet was resuspended in ice-cold buffer containing 50 mM Tris–HCl (pH 7.6), 2 mM dithiothreitol and 2 mM EGTA. This washing process was repeated twice. Finally, the pellet was resuspended with the membrane buffer at a concentration of approximately 1 mg protein/ml. The standard reaction mixture contained 50 mM Tris–HCl (pH 7.6), 1 mM EGTA, 5 mM MgCl2, 1 mM ATP containing [^{32}P]α-ATP (106 cpm), 1 mM cAMP containing [^{3}H]8-cAMP (10,000 cpm), 5 mM phosphocreatine, 250 mg/ml phosphocreatinase, 10 mM GTP and 30 mg of membrane protein in a final volume of 60 ml. Reactions were initiated by the addition of membrane preparation followed by

incubation for 10 min at 35°C. Reactions were terminated by the addition of 200 ml 0.5 M HCl, followed by boiling for 6 min and neutralization of the solution with 200 ml 1.5 M imidazole. Cyclic AMP formed during the incubation was extracted by using alumina columns and corrected for recovery of [^{3}H]cAMP. Protein concentration was determined by using the Bradford method with bovine serum albumin as the standard (Bradford, 1976).

2.3. Northern blot analysis

Total RNAs were prepared by the guanidium thiocyanate/phenol extraction method (Chomzyinski and Sacchi, 1987). Poly(A) + RNAs were isolated by the batch method using oligo (dT) cellulose. Poly (A) + RNA (10 mg) was electrophoresed on 1% agarose gel containing 2.2 M formaldehyde and transferred overnight onto Hybound N^+ membrane (Amersham) by capillary blotting. Probes specific for the four renal adenylyl cyclase (AC) isoforms AC 4, 5, 6 and 9 mRNA as well as for the vasopressin V_2 receptor mRNA and Gsα, Gαi-1, Gαi-2 and Gαi-3 mRNAs were prepared according to previously published procedures (Shen et al., 1997). The membranes were hybridized for 2 h at 65°C (in Rapid–Hyb buffer, Amersham) with 2×10^6 cpm/ml of each probe and washed in a sodium chloride/sodium citrate (SSC) solution (0.3 M NaCl, 0.03 M sodium citrate) containing 0.1% SDS (sodium dodecyl sulfate) detergent at room temperature for 20 min, followed subsequently by washes in 1:2 dilution of SSC/0.1% SDS at 65°C for 15 min and in 1:20 dilution of SSC/0.1% SDS at 65°C for 15 min, before being exposed to Kodak XAR film with intensifying screens at −80°C. The membranes were subsequently rehybridized with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe. For quantification autoradiographs were scanned using an InstantImager. Vasopressin V_2 receptor, AC and G protein mRNA levels were normalized to the expression of GAPDH. Exposure times were: 17 h for AC 4, 5 and 9, 5 h for AC 6, 18 h for vasopressin V_2 receptor, 2 h for Gsα, 14 h for Gαi-1, Gαi-2 and Gαi-3, and 1.5 h for GAPDH mRNAs.

2.4. Statistics

Values are expressed as means ± S.E.M. unless otherwise indicated, and paired or unpaired comparisons were made by using Student's paired and unpaired *t* tests, respectively.

3. Results

3.1. Determination of urine concentrating ability

Commonly used indicators of antidiuresis/diuresis include physiological variables such as daily fluid intake and output, and urinary Na^+ and K^+ concentrations.

3.1.1. V_2 agonist treatment of BDI rats

Five days of treatment of BDI rats with the vasopressin V_2 receptor agonist desamino-[Arg^8] vasopressin administered in the drinking water (i.e. self-regulating administrative procedure) resulted in a marked increase in urine concentration as indicated by the average 85% decrease in daily urine volume, from 125.5 ± 5.9 to 17.9 ± 1.1 ml ($P < 0.0001$), and the average 77% decrease in daily fluid intake from 148.4 ± 6.2 to 34.8 ± 2.9 ml ($P < 0.001$). Daily urine volume excreted by the control BDI rats did not alter significantly over the same period (160 ± 16.4 and 161.6 ± 14.6 ml) and neither did the fluid intake (179.3 ± 14.3 to 186.6 ± 11.2 ml).

3.1.2. V_2 antagonist treated Sprague–Dawley rats

In Sprague–Dawley rats, treatment with the vasopressin V_2 receptor antagonist SR was associated with a loss of concentrating ability as indicated by significantly lower urinary Na^+ and K^+ concentrations (153.6 ± 13.9 and 261.1 ± 18.4 mmol/l, respectively) compared with values determined in control untreated animals (255.8 ± 28.2 and 437.5 ± 54.6 mmol/l, respectively; both comparisons, $P < 0.005$).

3.2. Adenylyl cyclase activity

There were no differences between basal AC activity measured in desamino-[Arg^8] vasopressin treated and control BDI rats (17.8 ± 0.3 and 22.1 ± 1.7 pmol/min/mg protein, respectively) or in their vasopressin-stimulated AC activity at any of the concentrations used (see Fig. 1). Likewise, there were no differences between basal AC activity measured in SR 121463A-treated and control

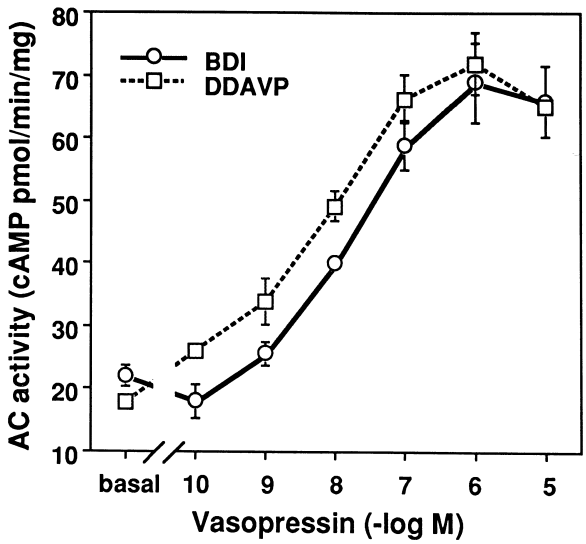

Fig. 1. Vasopressin-stimulated renal adenylyl cyclase (AC) activity in desamino-[Arg^8] vasopressin-treated (DDAVP) and untreated BDI (BDI) rats. Values are means (+S.E.M.) of triplicate determinations.

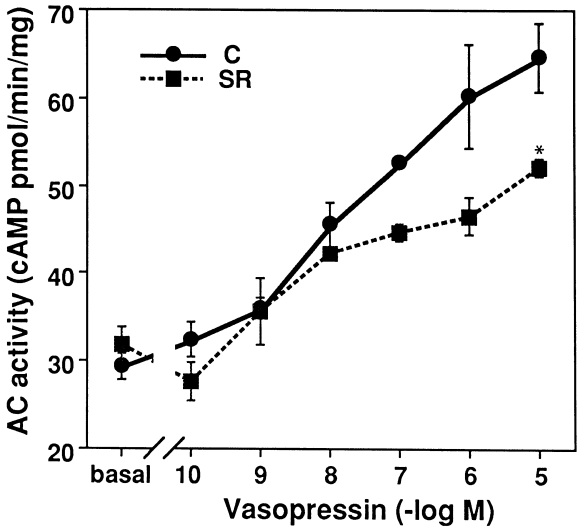

Fig. 2. Vasopressin-stimulated renal adenylyl cyclase (AC) activity in SR 121463A-treated (SR) and vehicle-treated control (C) Sprague–Dawley rats. Values are means (+S.E.M.) of triplicate determinations. $^{*}P < 0.05$.

Sprague–Dawley rats (29.4 ± 1.5 and 31.9 ± 2 pmol/min/mg protein, respectively) or in their vasopressin-stimulated AC activity at any of the concentrations used except the highest dose (see Fig. 2).

3.3. Northern blots

3.3.1. Adenylyl cyclase mRNA

Northern blots for AC 4, 5, 6 and 9 mRNAs obtained from the kidneys of desamino-[Arg^8] vasopressin treated and control BDI rats are shown in Fig. 3, together with changes for each AC mRNA expressed as ratios of the desamino-[Arg^8] vasopressin/control BDI values. AC 5, 6 and 9 mRNAs all increased with desamino-[Arg^8] vasopressin treatment (by 17, 22 and 11%, respectively) while AC 4 mRNA expression decreased by 17%. Northern blots for AC 4, 5, 6 and 9 mRNAs obtained from the kidneys of SR 121463A-treated and control rats are shown in Fig. 4, together with changes for the AC mRNAs expressed as ratios of the antagonist treated/control values. In contrast with the effects of the vasopressin V_2 receptor agonist, AC 5, 6 and 9 mRNAs were decreased by the antagonist treatment (by 22, 7 and 6%, respectively) while AC 4 mRNA expression was increased by 14.5%. None of these changes in mRNA was pronounced, however, suggesting that there was relatively little effect of either treatment on any of the renal adenylyl cyclase isoforms.

3.3.2. V_2 receptor and G protein mRNAs

Desamino-[Arg^8] vasopressin treatment of BDI rats was associated with a 34% increase in vasopressin V_2 receptor mRNA expression compared with that of untreated animals (Fig. 5). While there was no difference in Gsα mRNA expression in the two BDI groups, two of the inhibitory G proteins were particularly raised (Gαi-2 by 54% and Gαi-3 by 57%) in the desamino-[Arg^8] vasopressin treated animals. Gαi-1 mRNA expression was moderately higher (by 18%) in the treated BDI rats. These changes contrast with those detected in the vasopressin V_2 receptor antagonist treated Sprague–Dawley rats (Fig. 6) which, compared with their untreated controls, were relatively small and opposite in direction to the changes observed in the desamino-[Arg^8] vasopressin treated BDI rats (cf. Fig. 5). For example, vasopressin V_2 receptor mRNA decreased (by 8%) in SR 121463A-treated rats, while it increased in desamino-[Arg^8] vasopressin treated BDI rats.

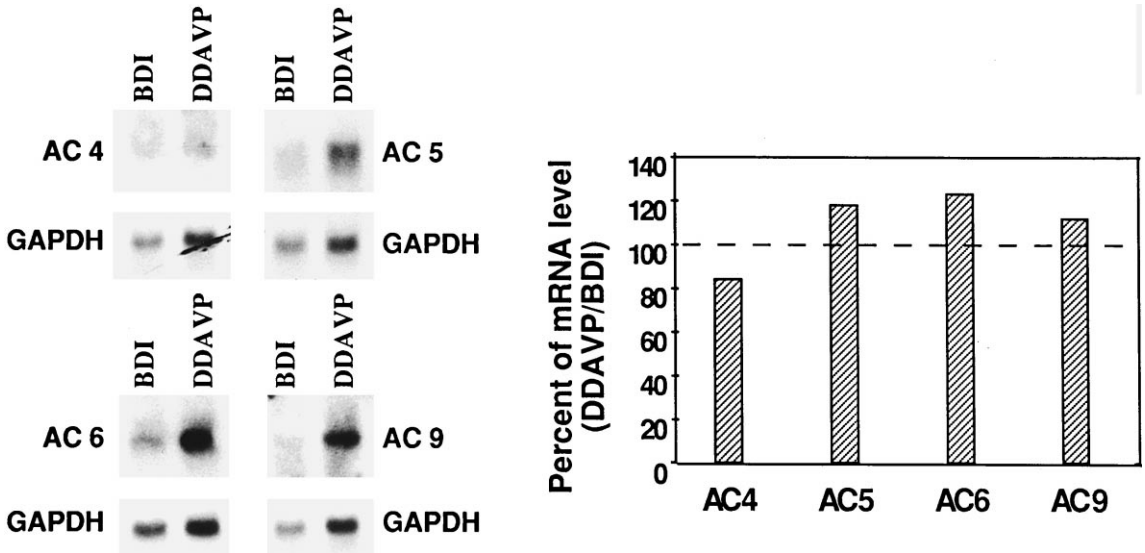

Fig. 3. Adenylyl cyclase expression (AC 4, 5, 6 and 9) in desamino-[Arg^8] vasopressin-treated and untreated BDI rat kidneys: Northern Blots and graph showing mRNA levels in the desamino-[Arg^8] vasopressin-treated rats relative to those detected in untreated BDI animals.

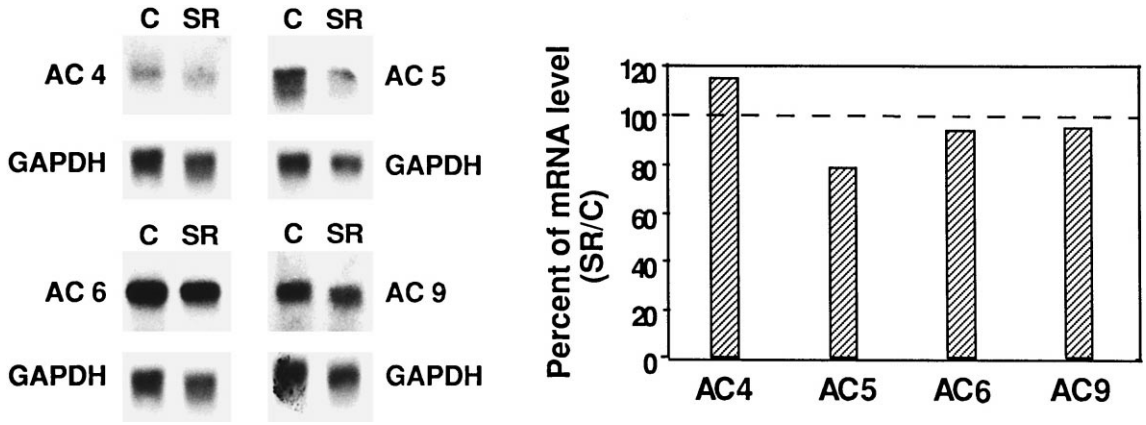

Fig. 4. Adenylyl cyclase expression (AC 4, 5, 6 and 9) in SR 121463A- and vehicle-treated (C) Sprague Dawley rat kidneys: Northern Blots and graph showing mRNA levels in the SR 121463A-treated rats relative to those detected in the vehicle-treated controls.

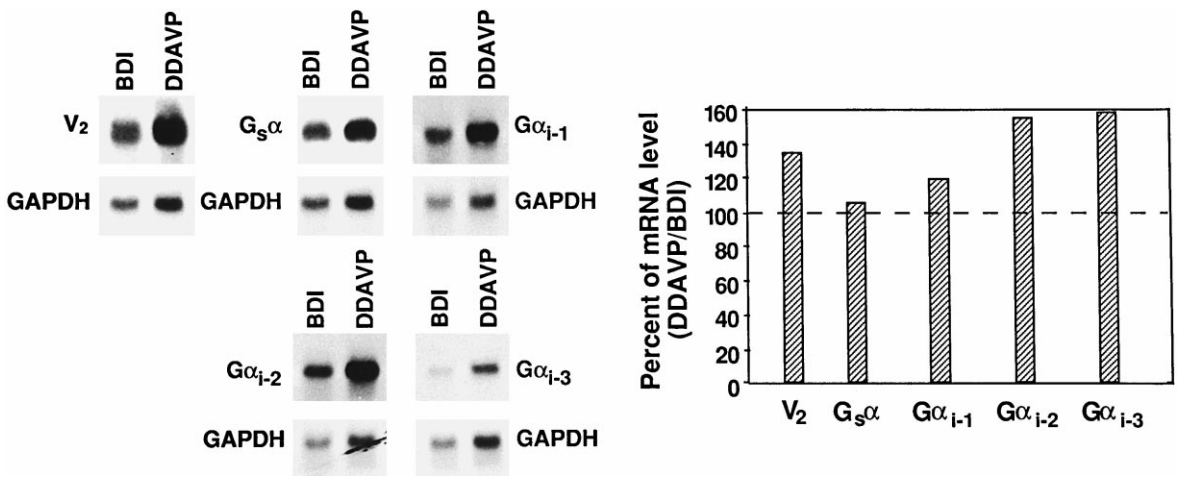

Fig. 5. Expression of vasopressin V_2 receptor and G-protein subunits (Gsα, Gαi-1, Gαi-2 and Gαi-3) in desamino-[Arg^8] vasopressin-treated and untreated BDI rat kidneys: Northern Blots and graph showing mRNA levels in the desamino-[Arg^8] vasopressin-treated rats relative to those detected in untreated BDI animals.

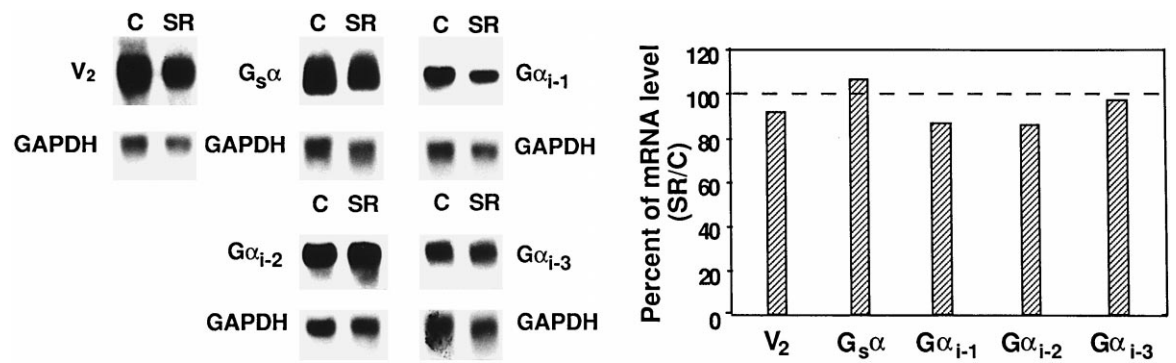

Fig. 6. Expression of vasopressin V_2 receptor and G-protein subunits (Gsα, Gαi-1, Gαi-2 and Gαi-3) in SR 121463A- and vehicle-treated (C) Sprague–Dawley rat kidneys: Northern Blots and graph showing mRNA levels in the SR 121463A-treated rats relative to those detected in the vehicle-treated controls.

4. Discussion

Increased or decreased concentrations of hormone or other receptor agonists are often associated with marked down-regulation and up-regulation of receptors, respectively. One particularly well described example is the regulation of β-receptors by adrenergic ligands. Impaired expression of specific receptors can also be related to the manifestation of disordered states. Thus the obese (ob/ob) mouse, which is resistant to the lipolytic effects of catecholamines, has been shown to have a massive 300-fold decrease in β_3-adrenergic receptors in adipose tissue (Collins et al., 1994).

Another example of altered receptor regulation is provided by the Brattleboro rat with hereditary hypothalamic diabetes insipidus, which lacks circulating vasopressin. However, paradoxically, renal vasopressin V_2 receptor numbers and mRNA expression are lower in these animals than in normal rats (Shen et al., 1997) whereas an up-regulatory effect might be expected. Furthermore, both vasopressin V_2 receptor numbers and mRNA are only between 25 and 30% reduced compared with those of their normal counterparts, these being surprisingly moderate reductions given the absence of circulating vasopressin. The decrease in vasopressin V_2 receptor expression following administration of the highly potent and selective vasopressin V_2 receptor antagonist SR 121463A to Sprague–Dawley rats also was small (a mere 8% lower than in untreated animals) even though urinary dilution is clearly present (Serradeil-Le Gal et al., 1996). Down-regulation of vasopressin V_2 receptor mRNA expression has also been reported in response to water deprivation. A 24 h dehydration, associated with a 13-fold increase in circulating plasma vasopressin concentrations, was associated with a reduction by only 33% of renal vasopressin V_2 receptor (but not V1a) receptor mRNA (Terada et al., 1993). A comparable 25% reduction in receptor numbers was reported in a human vasopressin V_2 receptor transfected fibroblast cell line sensitive to vasopressin in response to high concentrations of the hormone added to the medium (Birnbaumer et al., 1992). Furthermore, in the present study, restoration of urine concentrating ability in BDI rats was associated with a 34% increase in renal vasopressin V_2 receptor mRNA.

Thus all these results suggest that for this hormone–receptor system there is a pre-determined vasopressin V_2 receptor set-point which can only be varied within a relatively small range; neither the (chronic) total absence of circulating vasopressin on the one hand, nor the very raised vasopressin levels after severe dehydration on the other cause dramatic changes. A large reserve population of vasopressin V_2 receptor which is not activated by vasopressin (or desamino-[Arg^8] vasopressin), and which therefore is not involved in this regulatory process, has been inferred from early binding studies (Butlen et al., 1978). Thus the down-regulatory process seen in the present experiments may be more impressive for the vasopressin-sensitive pool of receptors. Interestingly, the water retention which is a characteristic of the raised circulating vasopressin concentrations of congestive heart failure has recently been shown to be associated with increased expression of the aquaporin-2 water channel (Nielsen et al., 1997). Similarly, the upregulation of aquaporin-2 in rats with chronic cardiac failure could be inhibited by the administration of a vasopressin V_2 receptor antagonist, which produced significant diuresis in these animals (Xu et al., 1997). Thus increases in circulating vasopressin concentrations produce increased antidiuretic activity via increased aquaporin-2 mRNA without any manifestation of receptor up-regulation.

The role played by the interaction of receptors with intracellular effector systems such as adenylyl cyclase–cyclic AMP in the control of receptor down- or up-regulation is currently the subject of much research. In the present study there were no marked differences in basal or vasopressin-stimulated total kidney cAMP generation between the desamino-[Arg^8] vasopressin treated BDI rats and their controls. Interestingly, both basal and maximal vasopressin-stimulated adenylyl cyclase activity has been reported to be lower in untreated BDI rats compared with rats of the parent Long–Evans strain (Shen et al., 1997). Consequently, an increase in cAMP generation had been expected in response to desamino-[Arg^8] vasopressin treatment of BDI rats, in parallel with a return of urine concentration to normal values. It is possible that total kidney adenylyl cyclase activity is a rather imprecise indicator of changes induced within the inner medullary collecting duct and papilla (Bia et al., 1979). Whether or not there are any changes in total renal adenylyl cyclase activity, it is clear that they cannot be very large, again suggesting that the vasopressin receptor–adenylyl cyclase system is remarkably stable in rats.

Four adenylyl cyclase isoforms (AC 4, 5, 6 and 9) are expressed in mammalian kidney, of which AC 6 is the major one (Hanoune et al., 1997). Expression of these isoforms has been studied in untreated BDI and Long-Evans rats, with reduced expression of all isoforms being detected in the vasopressin-deficient animals (Shen et al., 1997). Northern blots were carried out in the present study to determine whether the expression of any or all of the AC isoform mRNAs would increase in vasopressin V_2 receptor agonist-treated BDI rats and decrease in the vasopressin V_2 receptor antagonist-treated Sprague–Dawley rats, compared with their respective controls. Modest differences in mRNA expression of all four AC isoforms were detected in BDI rats in response to the desamino-[Arg^8] vasopressin treatment. Likewise, the changes in AC mRNA expression following treatment of the Sprague–Dawley rats with the vasopressin V_2 receptor antagonist were also small or minimal, and were opposite in direction to those for the desamino-[Arg^8] vasopressin treated BDI rats. The AC 6 isoform does not seem to be influenced by the presence or absence of vasopressin V_2 receptor–ligand

interaction any more than the other AC isoforms. It is particularly interesting to note that AC 4 mRNA expression is influenced by vasopressin in a direction opposite to that for all the other renal isoforms, decreasing with agonist binding and increasing with antagonist–receptor binding. The physiological significance of the various changes in mRNA for AC isoforms with receptor–ligand interaction remains to be elucidated.

The receptor–adenylyl cyclase interaction occurs through the participation of various G proteins in the cell membrane. There were no major differences between the levels of $Gs\alpha$ mRNA between untreated BDI rats and LE rats, while expression of the various Gi protein mRNAs is generally reduced in the vasopressin-deficient animals (Shen et al., 1997). The present results also fail to show that there were major changes in the mRNA expression of the G proteins, $Gs\alpha$ and Gi, in the antagonist-treated Sprague–Dawley rats compared with their untreated controls. However, while changes in $Gs\alpha$ mRNA were absent in the BDI rats treated with the vasopressin V_2 receptor-agonist desamino-[Arg^8] vasopressin, there was increased expression of Gi mRNA, particularly for $G\alpha$i-2 and $G\alpha$i-3 proteins. It is quite possible that these inhibitory G proteins play a role in the intracellular regulatory mechanism that provides the stabilizing influence on the vasopressin V_2 receptor–adenylyl cyclase system. Further experiments should clarify their role in the intracellular regulation of the signalling process.

In conclusion, following restoration of the urine concentrating ability of the BDI rat kidney with the vasopressin V_2 receptor agonist desamino-[Arg^8] vasopressin, there was an increase in receptor mRNA expression to levels detected in control normal LE rats, but very modest changes in mRNA expression for AC isoforms 4, 5, 6 and 9. While there was change in $Gs\alpha$ mRNA expression following desamino-[Arg^8] vasopressin treatment, there was a marked increased in the expression of $G\alpha$i-2 and $G\alpha$i-3 mRNA. In contrast, treatment of normal rats with a specific V_2 receptor antagonist had little effect on either vasopressin V_2 receptor, AC isoform or G protein mRNA expression. Unlike other hormone–receptor–effector systems, renal vasopressin V_2 receptor–adenylyl cyclase is robust and operates within a relatively narrow framework, there being very little evidence for major receptor down-regulation. The inhibitory G proteins, particularly $G\alpha$i-2 and $G\alpha$i-3, may be involved in the intracellular signal-stabilizing system.

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References

Aarons, R.D., Nies, A.S., Gal, J., Hegstrand, L.R., Molinoff, P.B., 1980. Elevation of b-adrenergic receptor density in human lymphocytes after propranolol administration. J. Clin. Invest. 65, 949–957.

Aiyar, N., Nambi, P., Crooke, S.T., 1990. Desensitization of vasopressin sensitive adenylate cyclase by vasopressin and phorbol esters. Cell. Signal. 2, 153–160.

Bia, M.J., Dewitt, S., Forrest, J.N., 1979. Dissociation between plasma, urine, and renal papillary cyclic AMP content following vasopressin and DDAVP. Am. J. Physiol. 237, F218–F225.

Birnbaumer, M., Antaramian, A., Themmen, A.P.N., Gilbert, S., 1992. Desensitization of the human V_2 vasopressin receptor. J. Biol. Chem. 267, 11783–11788.

Bohm, S.K., Grady, E.F., Bunnett, N.W., 1997. Regulatory mechanisms that modulate signalling by G-protein-coupled receptors. Biochem. J. 322, 1–18.

Bradford, M.M., 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye-binding. Anal. Biochem. 72, 248–254.

Butlen, D., Guillon, G., Rajerison, R.M., Jard, S., Sawyer, W.H., Manning, M., 1978. Structural requirements for activation of vasopressin-sensitive adenylate cyclase, hormone binding, and antidiuretic actions: Effects of highly potent analogues and competitive inhibitors. Mol. Pharmacol. 14, 1006–1017.

Chomzyinski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidium thiocyanate–phenol–chlorophorm extraction. Anal. Biochem. 162, 156–159.

Collins, S., Daniel, K.W., Rohifs, E.M., Ramkumar, V., Taylor, I.L., Gettys, T.W., 1994. Impaired expression and functional activity of the b3- and b1-adrenergic receptors in adipose tissue of congenitally obese (C57BL/6J ob/ob) mice. Mol. Endocrinol. 8, 518–527.

DiGiovanni, S.R., Nielsen, S., Christensen, E.I., Knepper, M., 1994. Regulation of collecting duct water channel expression by vasopressin in Brattleboro rat. Proc. Natl. Acad. Sci. 91, 8984–8988.

Dousa, T.P., Hui, Y.F.S., Barnes, L.D., 1975. Renal medullary adenylate-cyclase in rats with hypothalamic diabetes insipidus. Endocrinology 97, 802–807.

Glaubiger, G., Lefkowitz, R.J., 1977. Elevated beta-adrenergic receptor number after chronic propranolol treatment. Biochem. Biophys. Res. Commun. 78, 720–725.

Hanoune, J., Pouille, Y., Tzavara, E., Shen, T., Lipskaya, L., Miyamoto, N., Suzuki, Y., Defer, N., 1997. Adenylyl cyclases: Structure, regulation and function in an enzyme superfamily. Mol. Cell. Endocrinol. 128, 179–194.

King, L.S., Agre, P., 1996. Pathophysiology of the aquaporin water channels. Ann. Rev. Physiol. 58, 619–648.

Laycock, J.F., 1994. Desamino-8D-arginine vasopressin treatment of Brattleboro rats: Effect on sensitivity to pressor hormones. Eur. J. Pharmacol. 271, 193–199.

Lester, B.R., Sheppard, J.R., Burman, M., Somkuti, S.B., Stassen, F.L., 1985. Desensitization of LLC-PK1 cells by vasopressin results in receptor down-regulation. Mol. Cell. Endocrinol. 40, 193–204.

Mukherjee, C., Caron, M.G., Lefkowitz, R.J., 1976. Regulation of adenylate cyclase coupled beta-adrenergic receptors by beta-adrenergic catecholamines. Endocrinology 99, 347–357.

Nielsen, S., Terris, J., Andersen, D., Ecelbarger, C., Frokler, J., Jonassen, T., Marples, D., Knepper, M.A., Petersen, J.S., 1997. Congestive heart failure in rats is associated with increased expression and targeting of aquaproin-2 water channel in collecting duct. Proc. Natl. Acad. Sci. 94, 5450–5455.

Rajerison, R.M., Butlen, D., Jard, S., 1977. Effects of in vivo treatment with vasopressin and analogues on renal adenylate cyclase responsiveness to vasopressin stimulation in vitro. Endocrinology 101, 1–12.

Ravid, R., Swaab, D.F., Pool, C.W., 1985. Immunocytochemical localization of vasopressin-binding sites in the rat kidney. J. Endocrinol. 105, 133–140.

Serradeil-Le Gal, C., Lacour, C., Valette, G., Garcia, G., Foulon, L., Galindo, G., Bankir, L., Pouzet, B., Guillon, G., Barberis, C., Chicot, D., Jard, S., Vilain, P., Garcia, C., Marty, E., Raufaste, D., Brossard, G., Nisato, D., Maffrand, J.P., Le Fur, G., 1996. Characterization of SR 121463A, a highly potent and selective, orally active vasopressin V_2 receptor antagonist. J. Clin. Invest. 98, 2729–2738.

Shen, T., Suzuki, Y., Poyard, M., Miyamoto, N., Defer, N., Hanoune, J., 1997. Expression of adenylyl cyclase mRNAs in the adult, developing and Brattleboro rat kidney. Am. J. Physiol. 273, C323–C330.

Terada, Y., Tomita, K., Nonoguchi, H., Yang, T., Marumo, F., 1993. Different localization and regulation of two types of vasopressin receptor messenger RNA in microdissected rat nephron segments using reverse transcription polymerase chain reaction. J. Clin. Invest. 92, 2339–2345.

Xu, D.-L., Martin, P.-Y., Ohara, M., St. John, J., Pattison, T., Meng, X., Morris, K., Kim, J.K., Schrier, R.W., 1997. Upregulation of aquaporin-2 water channel expression in chronic heart failure rat. J. Clin. Invest. 99, 1500–1505.

Yamamoto, T., Sasaki, S., Fushimi, K., Ishibashi, K., Yaoiya, E., Kawasaki, K., Marumo, F., Kihaha, I., 1995. Vasopressin increases AQP-CD water channel in apical membrane of collecting duct cells in Brattleboro rats. Am. J. Physiol. 268, C1546–C1551.