Somatostatin and α_2-adrenergic agonists selectively inhibit vasopressin-induced cyclic AMP accumulation in MDCK cells

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The effect of somatostatin and α_2-adrenergic agonists on cyclic AMP accumulation was examined in MDCK cells, grown in defined medium. These hormones inhibited vasopressin-induced cyclic AMP formation, without affecting either the basal or the glucagon- and prostaglandin E_2-stimulated level. Pretreating the cells with pertussis toxin, or incubating them with $MnCl_2$ at a low concentration reversed the effect of somatostatin and α_2-agonists. These results suggest that somatostatin and norepinephrine could selectively modulate the renal effect of vasopressin, via the inhibitory regulatory subunit (N_i) of adenylate cyclase.

(MDCK cell) *cyclic AMP* *Vasopressin* *Somatostatin* *Norepinephrine* *Pertussis toxin*

1. INTRODUCTION

Among the properties of the renal distal tubule epithelium, retained by the MDCK cell line, is hormonal responsiveness to vasopressin, glucagon and prostaglandin E_2 which stimulate intracellular cyclic AMP accumulation in these cells [1,2]. Furthermore, SRIF has been reported to inhibit vasopressin-induced cyclic AMP accumulation in the distal parts of the rat nephron [3]. A similar interaction between vasopressin and α_2-adrenergic agonists was recently described in the rat collecting tubule [4,5].

The purpose of this study was to examine, in MDCK cells, the possible inhibition by SRIF and α_2-adrenergic agonists of vasopressin-, glucagon- and prostaglandin E_2-stimulated cyclic AMP formation, and to define further the mechanism of such an inhibition. The results showed that vasopressin-induced cyclic AMP generation was selectively inhibited by SRIF and norepinephrine whereas the response to glucagon and prostaglandin E_2 was unaffected. This inhibition occurred likely through stimulation of the N_i subunit of adenylate cyclase.

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Abbreviations: SRIF, somatotropin release-inhibiting factor (somatostatin); MDCK, Madin-Darby canine kidney; IBMX, 3-isobutyl-1-methylxanthine; BSA, bovine serum albumin; IAP, islet-activating protein (pertussis toxin); HBSS, Hanks' balanced salt solution

2. MATERIALS AND METHODS

2.1. *Materials*

Arginine-vasopressin, prostaglandin E_2, L-norepinephrine bitartrate salt, DL-propranolol HCl, IBMX, BSA and indomethacin were from Sigma (St. Louis, MO), glucagon from Novo (Paris), SRIF from Clin-Midy (Paris) and clonidine HCl from Boehringer Ingelheim (FRG). Cell culture media were from Flow Labs (England), plastic ware from Falcon Labware (Oxnard, CA) and carrier-free $Na^{125}I$ from Amersham (England). IAP was a generous gift from Dr F. Maigré (Institut Pasteur, Paris).

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2.2. *Methods*

MDCK cells (passages 69–74) were grown in serum-free medium [6] and subcultured once weekly. For cyclic AMP determination, MDCK cells were grown to confluence (3–4 days; $\simeq 5 \times 10^5$ cells/well) in 24-well culture dishes. All steps were performed at 37°C. The cells were washed twice with 1 ml buffer (HBSS, 1 mg/ml BSA, 20 mM Hepes), preincubated for 10 min in buffer containing 0.5 mM IBMX, and incubated for 5 min in fresh buffer containing hormones and drugs to be tested. Cyclic AMP was extracted as in [4] and measured, after acetylation [7], by radioimmunoassay [8,9]. Results are expressed as pmol cyclic AMP/culture well, each well containing 55–60 μg cell protein [10].

3. RESULTS

3.1. *Effects of SRIF on hormone-induced cyclic AMP accumulation*

The hormonal responsiveness of MDCK cells is shown in fig.1. 1 μM vasopressin, glucagon and prostaglandin E_2 induced a 6-, 8- and 11-fold increase in cyclic AMP content, respectively, whereas isoproterenol induced no significant stimulation. Vasopressin and glucagon had additive effects. SRIF (fig.2) affected neither the basal cyclic AMP value (1.8 ± 0.3 vs 1.9 ± 0.2 pmol cAMP/well, without and with SRIF, respectively; NS) nor glucagon- (9.1 ± 0.4 vs 8.5 ± 0.6 pmol/well; NS) or prostaglandin-E_2- (8.1 ± 0.4 vs 8.4 ± 0.7 pmol/well; NS) stimulated cyclic AMP accumulation, whereas it significantly reduced the vasopressin-induced cyclic AMP generation (9.4 ± 0.4 vs 5.9 ± 0.6 pmol/well; $p < 0.01$) in a dose-dependent manner (fig.3).

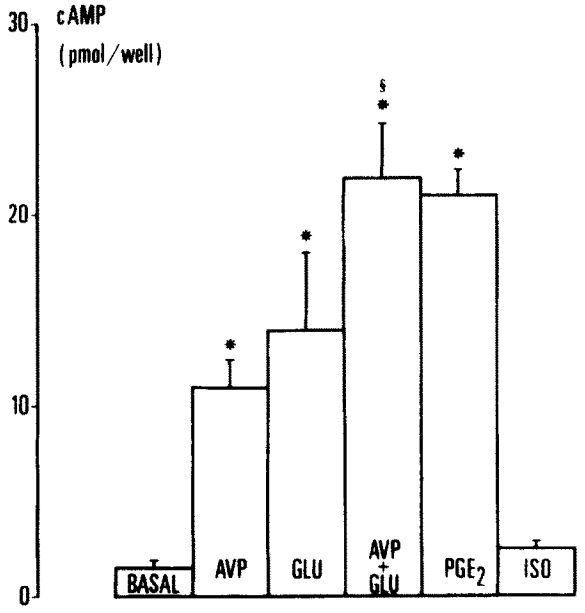

Fig.1. Effect of 1 μM vasopressin (AVP), glucagon (GLU), prostaglandin E_2 (PGE_2) and isoproterenol (ISO) on intracellular cyclic AMP (cAMP) accumulation. * Significantly different from the basal value, $p < 0.01$. § Significantly different from the value obtained with AVP or glucagon alone, $p < 0.01$.

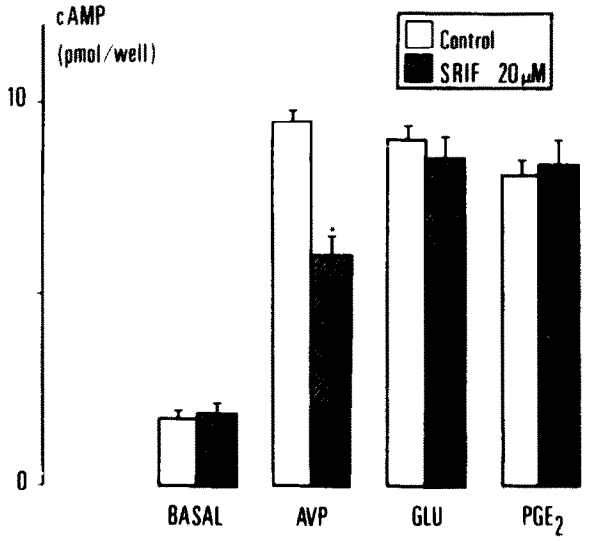

Fig.2. Effect of 20 μM SRIF on basal and 1 μM vasopressin- (AVP), glucagon- (GLU) and prostaglandin E_2- (PGE_2) stimulated intracellular cyclic AMP (cAMP) accumulation. * Significantly different from the value obtained without SRIF, $p < 0.01$.

3.2. *Effect of α_2-agonists on hormone-induced cyclic AMP accumulation (fig.4)*

10 μM norepinephrine did not affect intracellular cyclic AMP content under basal conditions (1.7 ± 0.2 vs 1.6 ± 0.2 pmol/well, without and with norepinephrine, respectively; NS), and after glucagon or prostaglandin E_2 stimulation. In contrast, norepinephrine dose-dependently inhibited vasopressin-induced cyclic AMP accumulation. A similar pattern was observed with the α_2-agonist clonidine.

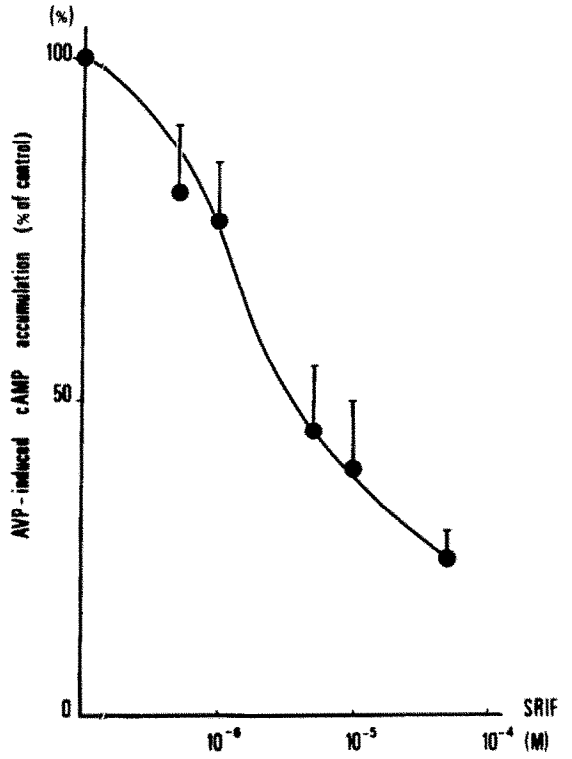

Fig.3. Effect of increasing concentrations of SRIF on 50 nM vasopressin- (AVP) stimulated cyclic AMP (cAMP) accumulation. The control value (without SRIF) was 8.5 ± 0.3 pmol/well (mean ± SE, $n = 6$). From 1 μM SRIF, values were significantly different from the control value, $p < 0.05$.

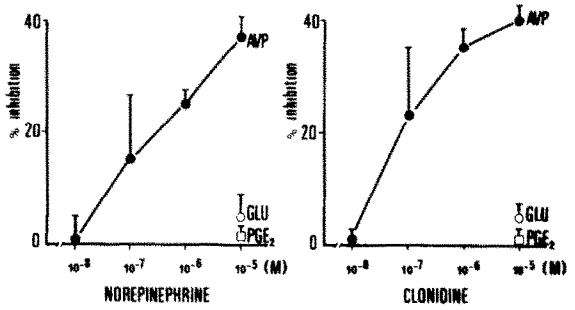

Fig.4. Effect of norepinephrine (left) and clonidine (right) on 50 nM vasopressin- (AVP), glucagon- (GLU), and prostaglandin E_2 (PGE_2) induced cyclic AMP (cAMP) accumulation. Control values (without norepinephrine or clonidine) were 8.7 ± 0.4, 7.5 ± 0.3 and 7.3 ± 0.3 pmol/well for vasopressin-, glucagon- and prostaglandin E_2-stimulated cyclic AMP accumulation, respectively (means ± SE, $n = 6$). The experiments with norepinephrine were performed in the presence of 10 μM propranolol, to block a possible β-adrenergic stimulation of cyclic AMP formation. From 0.1 μM norepinephrine and clonidine, values were different from the control value, $p < 0.05$.

3.3. *Effect of IAP and Mn^{2+} on SRIF and α_2-agonist inhibition*

18 h preincubation of MDCK cells with 250 ng/ml IAP reversed the inhibitory action of SRIF and norepinephrine on vasopressin-induced cyclic AMP accumulation (table 1). Incubation of

Table 1

Effect of pertussis toxin (IAP) on inhibition of intracellular cyclic AMP accumulation (pmol/well) induced by SRIF and norepinephrine

	IAP	Control	+ SRIF (20 μM)	+ norepinephrine (1 μM), propranolol (10 μM)
Basal	–	1.8 ± 0.23	1.8 ± 0.14	1.7 ± 0.12
	+	2.3 ± 0.64	2.5 ± 0.71	2.1 ± 0.42
Vasopressin (50 nM)	–	12.8 ± 1.39	8.9 ± 0.64[a]	8.3 ± 0.26[b]
	+	11.6 ± 0.59	10.8 ± 0.37[c]	9.8 ± 0.46[c]

[a,b] Significantly different from the control value, $p < 0.05$ and 0.02, respectively ($n = 6$)

[c] Significantly different from the homologous values without IAP, $p < 0.05$ ($n = 6$)

MDCK cells were preincubated for 18 h with 250 ng/ml IAP

Table 2

Effect of $MnCl_2$ (1 mM) on inhibition of intracellular cyclic AMP accumulation (pmol/well) induced by SRIF and clonidine

	$MnCl_2$	Control	+ SRIF (20 μM)	+ clonidine (1 μM)
Basal	–	1.4 ± 0.12	1.3 ± 0.10	1.4 ± 0.13
	+	1.6 ± 0.13	1.4 ± 0.31	1.7 ± 0.10
Vasopressin (50 nM)	–	17.6 ± 0.39	11.0 ± 0.39[a]	11.9 ± 0.20[a]
	+	24.2 ± 1.50[b]	23.4 ± 1.20[b]	21.5 ± 2.40[b]

[a] Significantly different from the control value, $p < 0.01$ ($n = 6$)
[b] Significantly different from the homologous values without $MnCl_2$, $p < 0.01$ ($n = 6$)

10 μM indomethacin was added to the medium during preincubation and incubation

the cells with 1 mM $MnCl_2$ led to a significant enhancement of vasopressin-induced cyclic AMP formation, and an almost complete reversion of the inhibitory effect of SRIF and clonidine (table 2).

4. DISCUSSION

The results showed that SRIF and α_2-adrenergic agonists inhibit vasopressin-induced, but not glucagon- or prostaglandin E_2-induced, cyclic AMP accumulation in MDCK cells, via stimulation of N_i, the inhibitory, regulatory component of adenylate cyclase.

In MDCK cells, α_1- and β-adrenergic-binding sites have been identified [11]. Our results, however, strongly suggest an α_2-receptor-mediated effect of catecholamines which could account, at least partly, for the antagonism between norepinephrine and vasopressin documented in vivo [12,13] and in vitro [14]. Cellular heterogeneity in MDCK cells was indicated by the additive effects of vasopressin and glucagon on cyclic AMP accumulation (fig.1), and likely accounted for the selective effect of SRIF and norepinephrine on vasopressin-induced, but not glucagon- or prostaglandin E_2-induced, cyclic AMP generation.

In other systems, inhibition of cyclic AMP accumulation by SRIF and norepinephrine was ascribed to a stimulation of N_i and could be reversed by IAP [15,16], which specifically inactivates N_i [17]. Our results (table 1) lead to a similar conclusion in MDCK cells. The absence of the reported increase by IAP [17] of hormone-stimulated cyclic AMP production might be the consequence of concomitant inhibition by IAP of prostaglandin synthesis [18,19]. Indeed, in the presence of indomethacin, manganese, which is known to inhibit selectively N_i [20] at low concentrations, enhanced the effect of vasopressin and blunted that of SRIF and clonidine.

As far as SRIF is concerned, its synthesis in toad urinary bladder and renal tubule [21] and rat glomeruli [22,23] is consistent with its role as a locally produced, modulating agent of vasopressin action, as evidenced in vivo [3,24] and in vitro [3,25,26] in both the amphibian bladder and the mammalian kidney.

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REFERENCES

[1] Rindler, M.J., Chuman, L.M., Shaffer, L. and Saier, M.H. (1979) J. Cell Biol. 81, 635–648.
[2] Lin, M.C. and Beckner, K. (1983) Curr. Top. Membranes Transp. 18, 287–315.
[3] Winkler, S.N., Torikai, S., Levine, B.S. and Kurokawa, K. (1982) Min. Electr. Metab. 7, 8–14.

[4] Chabardès, D., Montégut, M., Imbert-Teboul, M. and Morel, F. (1984) Mol. Cell. Endocrinol. 37, 263–275.
[5] Umemura, S., Marver, D., Smyth, D.D. and Pettinger, W.A. (1985) Am. J. Physiol. 249, F28–F33.
[6] Taub, M., Chuman, L., Saier, M.H. jr and Sato, G. (1979) Proc. Natl. Acad. Sci. USA 76, 3338–3342.
[7] Brooker, G., Harper, J.F., Terasaki, W.L. and Moylan, R.D. (1979) Adv. Cyclic Nucleotide Res. 10, 2–33.
[8] Steiner, A.L., Pagliara, A.S., Chase, L.R. and Kipnis, D.M. (1972) J. Biol. Chem. 247, 1114–1120.
[9] Friedlander, G., Chansel, D., Sraer, J., Bens, M. and Ardaillou, R. (1983) Mol. Cell. Endocrinol. 30, 201–214.
[10] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[11] Meier, K.E., Snavely, M.D., Brown, S.L., Brown, J.H. and Insel, P.A. (1983) J. Cell Biol. 97, 405–415.
[12] Klein, L.A., Liberman, B., Laks, M. and Kleeman, C.R. (1971) Am. J. Physiol. 221, 1657–1665.
[13] Fisher, D.A. (1968) J. Clin. Invest. 47, 540–547.
[14] Krothapalli, R.K. and Suki, W.N. (1984) J. Clin. Invest. 73, 740–749.
[15] Hewlett, E.L., Cronin, M.J., Mass, J., Anderson, H., Myers, G.A. and Pearson, R.D. (1984) Adv. Cyclic Nucleotide Protein Phosphorylation Res. 17, 173–182.
[16] Kurose, H., Katada, T., Amano, T. and Ui, M. (1983) J. Biol. Chem. 258, 4870–4875.
[17] Murayama, T. and Ui, M. (1983) J. Biol. Chem. 258, 3319–3326.
[18] Nakamura, T. and Ui, M. (1985) J. Biol. Chem. 260, 3584–3593.
[19] Hassid, A. (1983) J. Cell. Physiol. 116, 297–302.
[20] Seamon, K.B. and Daly, J.W. (1982) J. Biol. Chem. 257, 11591–11596.
[21] Bolaffi, J.L., Reichlin, S., Goodman, D.B.P. and Forrest, J.N. jr (1980) Science 210, 644–646.
[22] Kurokawa, K., Aponte, G.W., Fujibayashi, S. and Yamada, T. (1983) Kidney Int. 24, 754–757.
[23] Fujibayashi, S., Yamada, T. and Kurokawa, K. (1985) Mol. Cell. Endocrinol. 41, 263–267.
[24] Brautbar, N., Levine, B.S., Coburn, J.W. and Kleeman, C.R. (1979) Am. J. Physiol. 237, E428–E431.
[25] Forrest, J.N. jr, Reichlin, S. and Goodman, D.B.P. (1980) Proc. Natl. Acad. Sci. USA 77, 4984–4987.
[26] Roy, C. (1984) FEBS Lett. 169, 133–137.