Physiol. Res. 48: 513-517, 1999

RAPID COMMUNICATION

Effects of Immobilization Stress Combined with Water Immersion and Chronic Amphetamine Treatment on the Adenylyl Cyclase Activity in Rat Neurohypophysis

V. KLENEROVÁ, P. ŠÍDA, D. ENGLIŠOVÁ, J. STÖHR, E. NAZAROV, O. KAMINSKÝ, S. HYNIE

Department of Pharmacology, First Faculty of Medicine, Charles University, Prague, Czech Republic

Received June 30, 1999
Accepted October 3, 1999

Summary

Several papers have indicated the participation of cyclic AMP as a second messenger for the release of neurohypophysial hormones. Since very little is known about the effects of stress and drugs of abuse on this process, we studied the activity of adenylyl cyclase in the neurohypophyses after immobilization stress and chronic amphetamine treatment. Our findings indicate the involvement of cyclic AMP in the regulation of neurohypophysis as well as the increase in total adenylyl cyclase both after application of immobilization stress combined with water immersion and after chronic amphetamine treatment.

Key words

Neurohypophysis • Adenylyl cyclase • Stress • Amphetamine • Forskolin • G-regulatory protein

The neurohypophysis (neural lobe, posterior pituitary) is an organ which stores two important peptide hormones, oxytocin and vasopressin, that are synthesized in hypothalamic nuclei. Several stimuli are well known to activate the hypothalamo-neurohypophysial system (HNS) and to increase synthesis and blood levels of both hormones. However, the molecular mechanism of stimulation of hormone release from the neurohypophysis is not exactly known (Hatton 1990). There is some evidence that cyclic AMP participates in this process.

Neurohypophysis seems to have central regulation from the hypothalamus as well as direct regulation by a beta-adrenergic mechanism in pituicytes (Hatton 1988); these cells are probably activated not only by nerve stimulation from the CNS but also by circulating catecholamines (Luckman and Bicknell 1990), because the neurohypophysis is located outside the blood-brain-barrier (Hatton 1990).

Oxytocin and vasopressin are released under various physiological as well as pathological conditions (Cunningham and Sawchenko 1991, Herman and

Fax +420 2 293 894
E-mail physres@biomed.cas.cz http://www.lf2.cuni.cz/physiolres/physiolres.htm

Cullinan 1997). No sufficient data are available on the effects of various drugs of abuse on hormone release from the neurohypophysis. In any case there are indications that cyclic AMP system participates on the release of both neurohypophysial hormones (Anand-Srivastava 1988).

Due to a possible participation of cyclic AMP in the release of oxytocin and vasopressin, we decided to follow up the activity of adenylyl cyclase in the neurohypophysis. Since we recently found that some responses to drugs of abuse, such as amphetamine, are similar to the reactions that are found after exposure of the organism to stress (Klenerová 1998), we used both immobilization stress and chronic administration of amphetamine as stimuli to test whether they influence adenylyl cyclase activity in the neurohypophysis.

In this study we used male Wistar rats (Velaz, Konárovice, Czech Republic) of body weight 220-240 g which were kept in a special device (Flufrance, Vissous, France) for the whole duration of the experiment. This device enables the maintenance of constant temperature (21±1 °C), humidity (50-70 %), light regime (12L:12D cycle) and positive air pressure. High frequency noise was avoided. All animals were handled in accordance with the Declaration of Helsinki and guiding principles in the Care and Use of Animals (DHEW Publication, NHI 80-23).

Amphetamine (d-amphetamine sulphate, Sigma, St. Louis, USA) was administered intraperitoneally for 14 days with a single daily dose 4 mg/kg b.w. Control rats received saline solution in the same protocol as amphetamine-treated rats. Immobilization stress (IMO+C) used in this study was combined with the immersion of rats in water bath (22 °C) for 150 min (Klenerová and Šída 1994). The animals were killed by decapitation 24 h after the last amphetamine dose or immediately after the end of stress application.

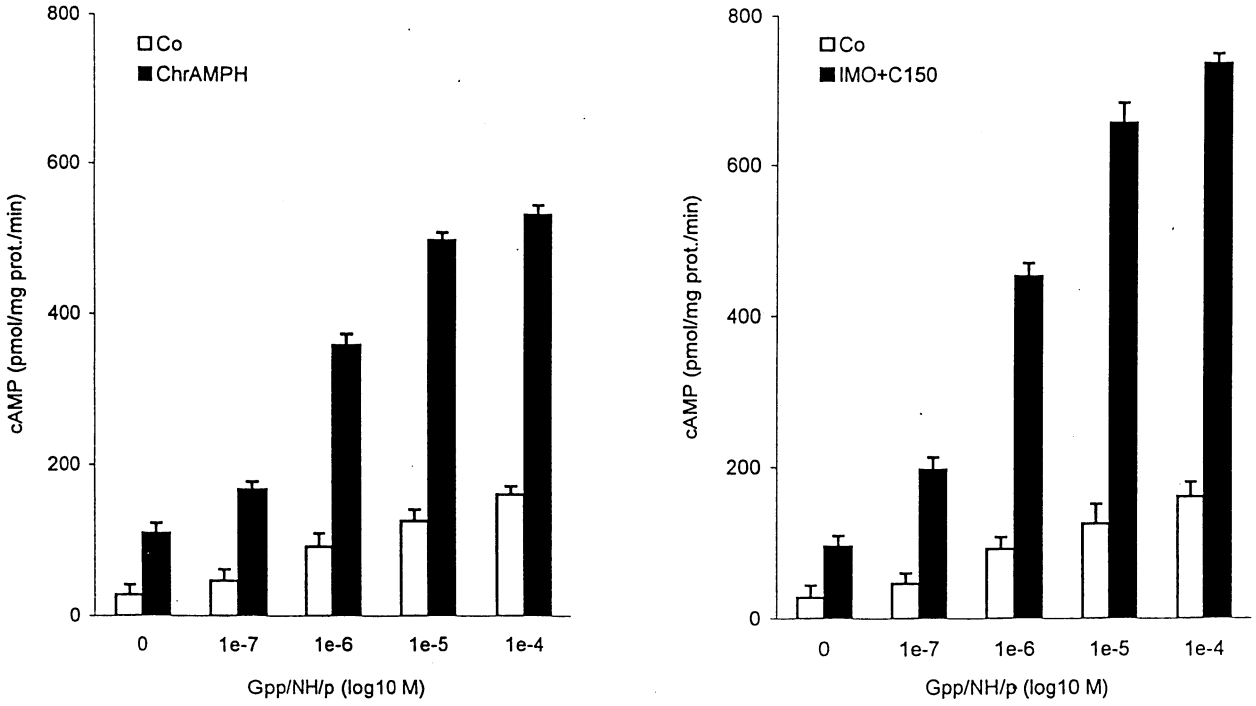

Fig. 1. *Activity of adenylyl cyclase in rat neurohypophysis stimulated by guanylylimidodiphosphate (Gpp/NH/p). Columns represent enzyme activity determined in triplicates in crude homogenates of pooled six neurohypophyses of each experimental group; mean values ±S.D. of triplicates. Co = appropriate control groups. Left: Enzyme activity in a group of rats stimulated by chronic amphetamine treatment (ChrAMPH). Right: Enzyme activity in a group of rats exposed to immobilization stress combined with water immersion (IMO+C).*

The neurohypophysis was separated from adenyhypophysis and the intermediate lobe was carefully removed under the microscope control. For all tests we collected and pooled six neurohypophyses for adenylyl cyclase assay. These samples were homogenized at 4 °C (ice bath) by an all glass tissue homogenizator with 10 strokes (Klenerová and Hynie 1974). This enzyme preparation was considered as a crude homogenate and

the enzyme activity was performed in triplicates. Adenylyl cyclase activity was estimated by using ^{32}P-α-ATP as a substrate (NEN Life Science Products, Boston, USA, specific activity was 800 Ci/mmol, about 10^6 cpm/assay, ATP was $5x10^{-5}$ M). After prepurification on Dowex X50 (Serva, Heidelberg, Germany) the product of the reaction was separated on aluminium oxide (Hynie 1990). The results are expressed in pmol of cyclic AMP produced per mg of protein (Lowry *et al.* 1951).

We followed the basal as well as stimulated adenylyl cyclase activity in all experimental groups. For the evaluation of enzyme activity related to the regulatory protein we used stimulation by non-hydrolysable analog of GTP guanylylimidodiphosphate (Gpp/NH/p) (Sigma, St. Louis, USA). Forskolin (Calbiochem, La Jolla, USA) stimulation was used for the assessment of total adenylyl cyclase activity. Both stimulants were added in a wide range of concentrations (indicated in figures).

Figure 1 presents the stimulatory effect of Gpp/NH/p on adenylyl cyclase activity after chronic amphetamine treatment (left) and immobilization stress (right). When compared to the control group, the exposure to chronic amphetamine caused an increase of both basal activities and the activity stimulated by Gpp/NH/p; this 2.5-fold increase was highly significant. The animals exposed to stress exhibited a similar response as rats treated with amphetamine. The increase of adenylyl cyclase activity stimulated by Gpp/NH/p was even higher than in the amphetamine group. These results clearly indicate a similar effect of both stimuli on neurohypophysial adenylyl cyclase activity.

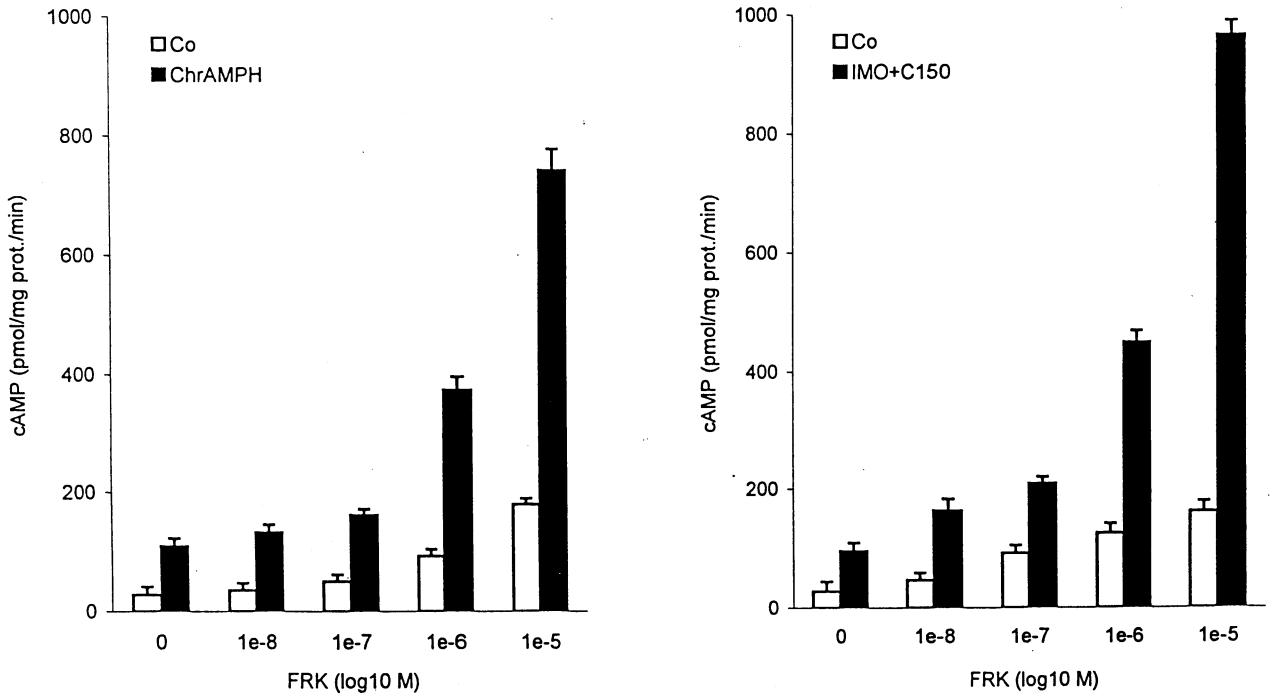

Fig. 2. *Activity of adenylyl cyclase in rat neurohypophysis stimulated by forskolin (FRK). Columns represent enzyme activity determined in triplicates in crude homogenates of pooled six neurohypophyses of each experimental group; mean values ± S.D. of triplicates. Co = appropriate control groups. Left: Enzyme activity in a group of rats stimulated by chronic amphetamine treatment (ChrAMPH). Right: Enzyme activity in a group of rat exposed to immobilization stress combined with water immersion (IMO+C).*

Figure 2 compares the stimulatory effects of forskolin on adenylyl cyclase activity after chronic amphetamine treatment (left) and immobilization stress (right). The results with forskolin, indicating the total adenylyl cyclase activity, are very similar to those obtained by using Gpp/NH/p as stimulator. However, the enhancement due to amphetamine treatment or immobilization is even higher than in the Gpp/NH/p group of rats. These data indicate the enhancement of total adenylyl cyclase activity after both stimuli.

We thus found significant changes in adenylate cyclase activity in the neurohypophysis after chronic application of amphetamine or immobilization stress. These changes clearly indicate the involvement of cyclic

AMP due to these potent stimuli that are probably mediated by beta-adrenergic mechanisms. Our work, which was focused on a single biochemical parameter, could not reveal any correlation between the activity of adenylyl cyclase and secretion of oxytocin and vasopressin. However, it provides a basis for speculations on this point. It is known that various stimuli which change the hormonal release from the neurohypophysis also induce morphological changes in pituicytes (Theodosis *et al.* 1998) that might be related to beta-adrenergic mechanisms and thus also to the cyclic AMP system. Of great importance are the findings of Lutz-Bucher *et al.* (1996), that the adenylyl cyclase activator PACAP (pituitary adenylate cyclase-activating polypeptide) releases both AVP and oxytocin and that these effects are mimicked by dibutyryl cyclic AMP. In the neurohypophysis it is not only PACAP, but also other neurotransmitters which participate in modulating the secretion of neurohypophysial hormones in an interactive way, mainly through the cyclic AMP system (Beagley and Hatton 1994).

Our results clearly indicate that extensive changes occur in adenylyl cyclase activity in the neurohypophysis after chronic amphetamine treatment and immobilization stress. These changes show intensive plasticity of this system that has been demonstrated by others who investigated other criteria of stimulus-secretion coupling of peptide hormones from the neurohypophysis (Hatton 1990). It seems of great importance that such divergent stimuli as amphetamine treatment and stress both produce changes in total adenylyl cyclase activity. Similar responses of this enzyme activity to these stimuli were also observed in rat frontal cortex and striatum (Klenerová *et al.* 1998). It would be desirable to ascertain whether the observed changes in adenylyl cyclase activity in the neurohypophysis are typical for amphetamine treatment or common to all CNS stimulating drugs that would suggest their action as stressors.

Acknowledgements

This work was supported by grants from the Ministry of Health of Czech Republic No. 4969-3 and by institutional support No. VZ CEZ 13 3/98: 1111 00001.

References

ANAND-SRIVASTAVA MB: Adenosine interaction with adenylate cyclase in rat posterior pituitary. *Mol Cell Endocrinol* **57:** 231-237, 1988.

BEAGLEY GH, HATTON GI.: Systemic signals contribute to induced morphological changes in the hypothalamo-neurohypophysial system. *Brain Res Bull* **33:** 211-218, 1994.

CUNNINGHAM ET, SAWCHENKO PE.: Reflex control of magnocellular vasopressin and oxytocin secretion. *Trends Neurosci* **14:** 406-411, 1991.

HATTON GI: Pituicytes, glia and control of terminal secretion. *J Exp Biol* **139:** 67-79, 1988.

HATTON GI: Emerging concepts of structure-function dynamics in adult brain: the hypothalamo-neurohypophysial system. *Prog Neurobiol* **34:** 434-504, 1990.

HERMAN JP, CULLINAN WE: Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci* **20:** 78-84, 1997.

HYNIE S: *Membrane Receptors and Transmembrane Signalling.* Rozpravy Československé akademie věd (Vol. 100, No. 6) Academia, Prague, 1990, pp 57-61.

KLENEROVÁ V: Cyclic AMP system as a common target for amphetamine and/or stress effects. In: *Mechanisms in Drug Abuse and Addiction Translation to Treatment.* Proc Third US/Central Europe Reg Mtg on Drug Dependence, Brno 1997, *NIDAINVEST, Bethesda, MD,* 1998, p 31.

KLENEROVÁ V, HYNIE S: The activity of adenylate cyclase, phosphodiesterase and protein kinase in the adenohypophyses in rats treated by chronic administration of estrogens in vivo. *Horm Metab Res* **6:** 208-213, 1974.

KLENEROVÁ V, ŠÍDA P: Changes in beta-adrenergic receptors in the neurohypophysis and intermediate lobe of rat hypophysis exposed to stress. *Physiol Res* **43:** 289-292, 1994.

KLENEROVÁ V, ŠÍDA P, HYNIE S: Different activity of adenylyl cyclase in prefrontal cortex in three rat strains; the effect of amphetamine. *Folia Biol* **44:** 133-136, 1998.

LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265-275, 1951.

LUCKMAN SM, BICKNELL RJ: Morphological plasticity that occurs in the neurohypophysis following activation of the magnocellular neurosecretory system can be mimicked in vitro by beta-adrenergic stimulation. *Neuroscience* **39**: 701-709, 1990.

LUTZ-BUCHER B, MONNIER D, KOCH B: Evidence for the presence of receptors for pituitary adenylate cyclase-activating polypeptide in the neurohypophysis that are positively coupled to cyclic AMP formation and neurohypophyseal hormone secretion. *Neuroendocrinology* **64:** 153-161, 1996.

THEODOSIS DT, EL MAJDOUBI M, PIERRE K, POULAIN DA: Factors governing activity-dependent structural plasticity of the hypothalamoneurohypophysial system. *Cell Mol Neurobiol* **18:** 285-298, 1998.

Reprint requests

Assoc. Prof. V. Klenerová, M.D., D.Sc., Department of Pharmacology, First Faculty of Medicine, Charles University, Albertov 4, CZ-128 00 Prague 2, Czech Republic.