

SHORT COMMUNICATION

EFFECT OF GLUCOCORTICOID DEXAMETHASONE ON CYCLIC AMP FORMATION STIMULATED BY PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) IN THE CEREBRAL CORTEX AND HYPOTHALAMUS OF CHICK

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Effect of glucocorticoid dexamethasone on cyclic AMP formation stimulated by pituitary adenylylase-activating polypeptide (PACAP) in the cerebral cortex and hypothalamus of chick. J.B. ZAWILSKA, J.Z. NOWAK. Pol. J. Pharmacol., 2001, 53, 39–45.

In this study we tested in chicks the effects of acute and chronic *in vivo* treatment with a glucocorticoid dexamethasone (4 mg/kg, *ip*) on PACAP-stimulated cyclic AMP formation in [³H]adenine-prelabeled slices of the hypothalamus and cerebral cortex. PACAP (1–100 nM) concentration-dependently stimulated cyclic AMP formation in both brain regions of chick. In acute experiments, dexamethasone (single dose)-injected chicks were killed after 2, 24 and 48 h; while in chronic experiment the glucocorticoid was given once daily for 12 days and the animals were killed 48 h after the last injection. The ability of PACAP to stimulate cyclic AMP formation in the hypothalamus and cerebral cortex was similar in vehicle-treated (control) and dexamethasone-treated animals, with the exception of the nucleotide response to 100 nM of the peptide in both brain regions, which was significantly larger in the group of chicks killed 48 h after the administration of the single steroid dose.

Key words: *dexamethasone, PACAP, cyclic AMP, brain, chick*

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INTRODUCTION

Glucocorticoid hormones are known to affect different aspects of neuronal activity, including receptor-mediated responses [4, 5, 16, 28]. One of such responses involves the generation of cyclic AMP stimulated by several neuroregulators, such as noradrenaline (a classic neurotransmitter), prostaglandin E₁ and E₂, or adenosine, and these processes can be affected by glucocorticoids in a positive (enhancement) and negative (inhibition) manner, both in the brain tissue and in a variety of cultured cell lines [6–9, 13, 14, 30].

A positive and negative regulation of cyclic AMP formation by adrenal steroids has also been reported when a potent adenylyl cyclase activator, i.e. vasoactive intestinal peptide (VIP) was applied. The former type of the effect (finally leading to an enhancement of the VIP action) was observed in the cerebral cortical slices of rats chronically treated with dexamethasone [6], whereas the latter one (resulting in an inhibition of the VIP effect) occurred in the rat pituitary cells in culture after the addition of either dexamethasone or corticosterone to the incubation milieu [25].

The cyclic AMP response evoked by VIP results from the peptide interaction with two kinds of adenylyl cyclase-linked receptors, known earlier as VIP₁ and VIP₂ receptors [23]. Only recently these receptors were renamed to VPAC₁ and VPAC₂ receptors, as it appeared that they are also targets for a VIP-like peptide called the pituitary adenylyl cyclase-activating polypeptide (PACAP). This novel peptide, in addition to its interaction with VPAC₁ and VPAC₂ receptors, acts also on adenylyl cyclase-coupled PACAP-specific receptors designated as PAC₁ receptors [2, 10, 17, 18, 23, 29]. Literature data indicate that PACAP may play a role of a physiological regulator of various neuronal and endocrine processes in different species (e.g. [1, 15, 23, 29]). Although the PACAP-stimulated accumulation of cyclic AMP in cultured aortic smooth muscle cells was affected by dexamethasone [13], it is unknown whether the PAC₁ receptor-mediated cyclic AMP effects in the central nervous system (CNS) are also subject to glucocorticoid action, and this study was aimed at bridging this gap.

MATERIALS and METHODS

Animals

White male leghorn chicks (*Gallus domesticus*) were obtained on the day of hatching from the local hatchery and kept in warmed brooders with standard food and water available *ad libitum*, for two–three weeks before use. The animals were kept under a 12 h light/12 h dark lighting schedule (lights on between 21.30 and 09.30; light intensity near the floor of the animals' room was about 150 lux). In acute experiments chicks received intraperitoneal (*ip*) injections of demaxethasone (4 mg/kg) or vehicle (0.3 ml/animal), always between 08.30 and 09.00. Chicks were killed by decapitation 2, 24 or 48 h after the injection. In chronic experiments animals received *ip* injections of dexamethasone and vehicle once daily for 12 days and were killed 48 h after the last injection. Their brains were quickly removed, cerebral cortex (without white matter) and hypothalamus were isolated, and processed for biochemical measurements. The experiments were performed in accordance with the Polish governmental regulations concerning experiments on animals and rules followed at the Department of Biogenic Amines.

Assay of cyclic AMP formation

Each experiment was carried out on the hypothalamic tissue pooled from three animals and cerebral cortex (devoid of white matter) of two animals, and repeated 3–4 times. Cross-chopped slices (250 μm; prepared with McIlwain tissue chopper) of the selected brain regions were suspended in cold, O₂/CO₂ (95:5) gassed, glucose-containing modified Krebs-Henseleit medium (containing 118 mM NaCl, 5 mM KCl, 1.3 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, and 11.7 mM D-glucose; pH 7.4). The formation of [³H]cyclic AMP in [³H]adenine prelabeled tissues was assayed according to Shimizu et al. [27]. The formed [³H]cyclic AMP was isolated by a sequential Dowex-alumina chromatography according to Salomon et al. [26]. The results were individually corrected for a percentage recovery with the aid of [¹⁴C]cyclic AMP added to each column system prior to the nucleotide extraction. The accumulation of cyclic AMP during a 10-min stimulation period was assessed as a per cent of the conversion of [³H]ade-

nine to [^3H]cyclic AMP. Details of the whole procedure were described by us earlier [19, 21].

Chemicals

The following substances were used: PACAP₃₈ (A-1439) and dexamethasone supplied by Sigma (St. Louis, MO, USA); [^3H]adenine (specific activity 26.9 Ci/mmol) and [^{14}C]cyclic AMP (specific activity 52.3 mCi/mmol) purchased from DuPont New England Nuclear (Bad Homburg, Germany). Other chemicals were of analytical purity and were obtained mainly from Sigma (St. Louis, MO, USA).

Statistical analysis

Data were expressed as means \pm SEM, and they were analyzed by one-way analysis of variance followed by Newman-Keuls test, using GraphPad software.

RESULTS and DISCUSSION

The presence of PACAP-containing neurons and PACAP-sensitive cyclic AMP-generating system has recently been demonstrated for the avian brain [19, 20, 22]. In line with the previous findings [19], PACAP₃₈ (10^{-9} – 10^{-7} M) concentration-dependently stimulated cyclic AMP formation in the

hypothalamus and cerebral cortex of chick. Although the observed effects in these two brain regions were clearly expressed and reproducible, the results need a comment regarding their possible dual interpretation (depending on the way of presentation). Comparing the data obtained in the vehicle-treated chicks, it should be noticed that the PACAP-evoked effects are either decisively larger in the hypothalamus than in cerebral cortex (1049% vs. 249%), when the results are expressed as a percent of respective control, or really comparable in both structures (i.e. 7.30 vs. 6.10), while considering the values showing net increases in the cyclic AMP response (i.e., differences between PACAP-stimulated and basal values) (Tab. 1). In our opinion the data presented as “net increases” give a more realistic insight into the phenomenon under the study, thus this way of presentation of the findings was used.

Acute treatment of chicks with dexamethasone (4 mg/kg, *ip*) did not significantly influence the PACAP-evoked cyclic AMP responses in the hypothalamus and cerebral cortex of the animals killed 2 and 24 h after the steroid injection. However, in chicks treated once with dexamethasone and killed 48 h after the drug application, the effects produced by 100 nM PACAP (and not by the peptide used at concentrations of 1, 10 and 30 nM) in the two brain regions were significantly larger than those

Table 1. PACAP-stimulated cAMP formation in the chick brain

Experimental series No.	Basal	PACAP	% Basal	PACAP – Basal
	% conversion			% conversion
CEREBRAL CORTEX				
1	2.80	9.38	235	6.58
2	2.59	7.53	190	4.94
3	2.52	8.70	245	6.18
4	2.06	8.76	325	6.70
mean	2.49 \pm 0.15	8.59 \pm 0.39	249 \pm 28	6.10 \pm 0.40
HYPOTHALAMUS				
1	0.74	7.21	874	6.47
2	0.68	8.85	1200	8.17
3	0.45	7.29	1520	6.84
4	1.28	8.99	602	7.71
mean	0.79 \pm 0.17	8.08 \pm 0.48	1049 \pm 199	7.30 \pm 0.39

Results are taken from Fig. 1 and Fig. 2, and represent mean values for basal and 100 nM PACAP-evoked stimulations of cAMP formation in vehicle-treated animals. Numbers of experimental series refer to respective values in A, B, C and D shown in the figures

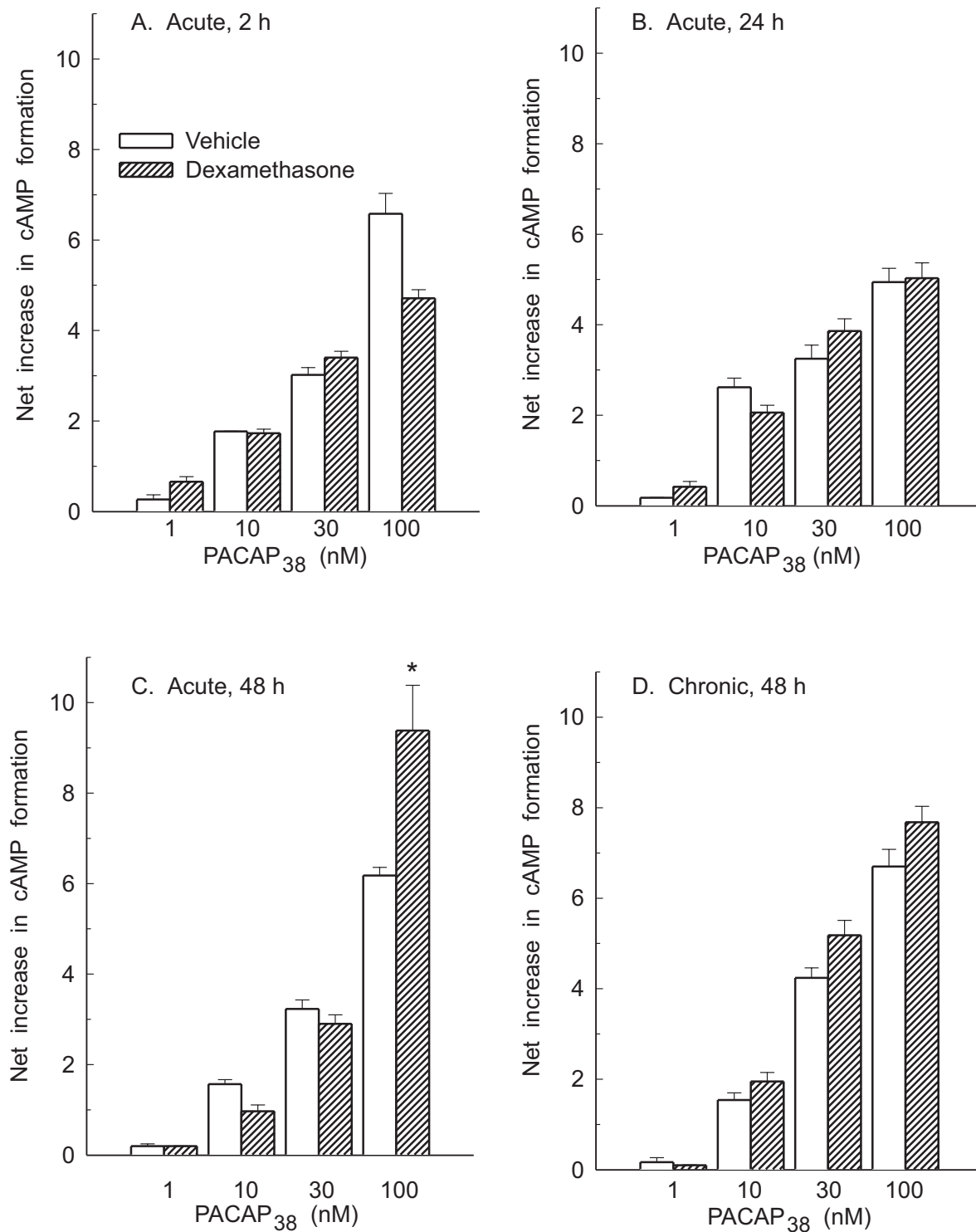


Fig. 1. Effects of acute and prolonged treatment with dexamethasone on the PACAP₃₈-evoked increases in cyclic AMP formation in the chick hypothalamus. The animals received dexamethasone (4 mg/kg, *ip*) or vehicle acutely (A-C) or once daily for 12 days (D), and they were killed at times indicated in the figures. Results are expressed as net increases in cAMP formation and represent means \pm SEM values. The number of experiments was: A, 8-16; B, 7-9; C, 8-12; D, 20-23. * $p < 0.05$ vs. vehicle-treated control

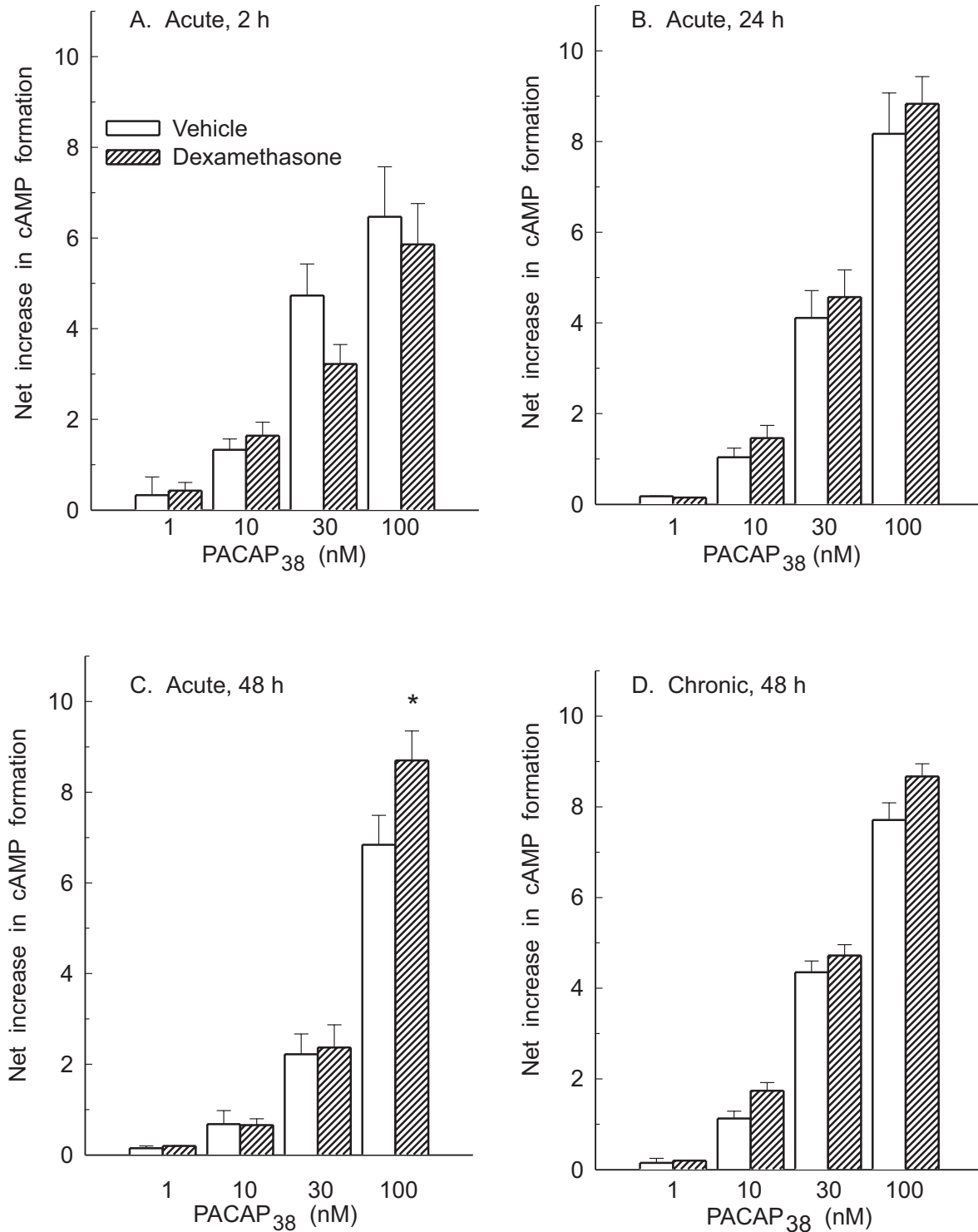


Fig. 2. Effects of acute and prolonged treatment with dexamethasone on the PACAP₃₈-evoked increases in cyclic AMP formation in the chick cerebral cortex. The animals received dexamethasone (4 mg/kg, *ip*) or vehicle acutely (A-C) or once daily for 12 days (D), and they were killed at times indicated in the figures. Results are expressed as net increases in cAMP formation and represent means \pm SEM values. The number of experiments was: A, 6-19; B, 8-11; C, 6-28; D, 17-21. * $p < 0.05$ vs. vehicle-treated control

observed in the control, vehicle-treated birds (Fig. 1C and Fig. 2C). Interestingly, these larger effects of PACAP obtained in acute (48 h) experiments were not observed in chicks receiving chronically dexamethasone (4 mg/kg, *ip*, once daily for 12 days) and killed 48 h after the last injection of the steroid (Fig. 1D and Fig. 2D).

A general impression coming up from the results of our study is that the activity of the PACAP-sensitive cyclic AMP-generating system(s) of the chicken brain does not seem to be under major regulatory control by glucocorticoids. Although we did observe some significant changes in the hypothalamus (Fig. 1C) and cerebral cortex (Fig. 2C), they were seen only in one experimental variant (acute treatment with dexamethasone and measurement 48 h later) and refer only to one concentration of the peptide, which makes their interpretation rather difficult. It should be noted, however, that our experimental protocol did not include the presence of an inhibitor of phosphodiesterase activity in the incubation medium. Hence, we cannot neglect a possibility that treatment with dexamethasone, by influencing the activity of either adenylyl cyclase or phosphodiesterase, a cyclic AMP synthesizing and catabolizing enzyme, respectively, or both, might affect the dynamics of the whole cyclic AMP system, the changes of which may be hardly detectable by measuring the steady-state level of the nucleotide. Further research is required for verification of such a possibility, especially in light of the fact that it has earlier been suggested that dexamethasone may decrease cyclic AMP catabolism in PC18 cells in culture [30].

Several experimental data indicate that mechanism(s) underlying the glucocorticoid-induced effects on cyclic AMP generation is evidently complex, and likely multifactorial. For example, the addition of corticosterone to the incubation medium decreased the forskolin-stimulated cyclic AMP production in rat cerebral cortical slices, whereas chronic injection of corticosterone or dexamethasone in rats led to increases in the forskolin-driven cyclic AMP response [3, 6]. The observed changes were most probably not related to a direct action of the chronically-given glucocorticoid on adenylyl cyclase, as the enzyme activity (when measured in brain homogenate) was similar in control and dexamethasone-treated rats [6]. Moreover, the forskolin-driven cyclic AMP accumulation was potentiated by dexamethasone in the cultured PC18 cells

[30] and inhibited in the cultured aortic smooth muscle cells [13]. In addition to that, the forskolin-stimulated adenylyl cyclase activity was also inhibited by dexamethasone in membrane preparation of the cultured aortic smooth muscle cells [13].

Earlier papers [11, 12, 24, 25] presented evidence suggesting the existence of a functionally important interaction between glucocorticoids and VIP in rats. The authors [24] concluded that “..the adrenal steroid environment is essential for the full expression of VIP activity on central and neuroendocrine function”. A recently reported inhibition of the PACAP-evoked cyclic AMP accumulation by dexamethasone in cultured aortic smooth muscle cells [13] could extend the validity of the above conclusion to PACAP. Yet, in these cells the glucocorticoid also inhibited the cyclic AMP responses to other vasoactive agents such as prostaglandin E₂ and carbacyclin, a stable analog of prostacyclin [13], suggesting that adrenal steroids may affect the cyclic AMP system(s) by itself, irrespective of the receptor type being under activation.

In summary, the results of the experiments performed in chicks, where the PACAP-induced cyclic AMP effects in the hypothalamus and cerebral cortex are mediated *via* the PAC₁-type receptor [19], indicate that a synthetic glucocorticoid dexamethasone given either acutely or chronically does not modify to a major extent the PAC₁-receptor-mediated activation of cyclic AMP accumulation in the CNS. Whether this conclusion is true for other vertebrates remains at present an open question.

Acknowledgments. We would like to thank Mrs. Teresa Kwapisz for her excellent technical assistance. This work was supported by the grant No. 502-13-603 from the Medical University of Łódź, Poland.

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Received: December 20, 2000; in revised form: January 22, 2001.