JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2001, 52, 4, 795—809

www.jpp.krakow.pl

J. BUGAJSKI, R. GŁÓD, A. GĄDEK-MICHALSKA, *A. J. BUGAJSKI

INVOLVEMENT OF CONSTITUTIVE (COX-1) AND INDUCIBLE CYCLOOXYGENASE (COX-2) IN THE ADRENERGIC-INDUCED ACTH AND CORTICOSTERONE SECRETION

Department of Physiology, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland, *Department of Pathophysiology, Jagiellonian University Medical College, Cracow, Poland

The involvement of prostaglandins synthesized by constitutive (COX-1) and inducible cyclooxygenase (COX-2) in central stimulation of the hypothalamic-pituitary-adrenal (HPA) axis by adrenergic receptor agonists was investigated in conscious rats. COX-1 and COX-2 inhibitor, piroxicam (0.02 and 0.2 μg) and compound NS-398 (0.01 and 0.1 μg), respectively, were given intracerebroventricularly (i.c.v.) 15 min prior to i.c.v. adrenergic receptor agonists: phenylephrine (30 μg) and clonidine (10 μg), an α_1- and α_2-adrenergic agonist, and isoprenaline (20 μg) a non-selective β-adrenergic agonist and clenbuterol (10 μg) a selective β_2-adrenergic agonist. Piroxicam and NS-398 considerably and dose-dependently reduced the phenylephrine-induced increase in ACTH and corticosterone secretion. Pretreatment with piroxicam and NS-398 markedly impaired the clonidine-evoked ACTH and corticosterone secretion. Piroxicam moderately diminished the isoprenaline-elicited increase in ACTH and corticosterone, while NS-398 did not markedly alter ACTH secretion. The clenbuterol-induced ACTH and corticosterone responses were considerably impaired by pretreatment with piroxicam, and slightly less potently by NS-398. These results indicate that in central structures involved in regulation of the HPA axis both constitutive and inducible cyclooxygenase are present under normal conditions in rats. These isoenzymes are significantly involved in the stimulatory signaling transduced by postsynaptic α_1-adrenergic receptors and, to a lesser extent, by α_2-adrenergic receptors. Both isoenzymes affect moderately the stimulatory action of a non-selective β-adrenergic agonist on ACTH and corticosterone secretion. COX-1 participates considerably and COX-2 markedly in the potent stimulatory action of selective β_2-adrenergic receptors on HPA axis.

K e y w o r d s: *constitutive cyclooxygenase (COX-1), inducible cyclooxygenase (COX-2), prostaglandins, adrenergic receptors, hypothalamic-pituitary-adrenal axis, ACTH, corticosterone*

INTRODUCTION

The major rate-limiting enzymes in prostaglandin synthesis are the cyclooxygenases (1). It was initially accepted that under basal conditions

prostaglandins are synthesized by constitutive cyclooxygenase (COX-1). COX-1 is distributed in neurons throughout the brain, and it is most prevalent in forebrain, where prostaglandins may be involved in complex integrative function (1). Presence of inducible cyclooxygenase (COX-2) was detected in cells of various tissues under physiological conditions, but it expression can be considerably increased during inflammation or following exposure to mitogenic stimuli (2—4). Recent studies have shown that COX-2 is also expressed constitutively in certain parts of the CNS, cortex, hippocampus, hypothalamus, and spinal cord (5, 6). Although in the rat brain mRNA of both COX isoenzymes is expressed constitutively, COX-2 is the predominant isoform. Under physiological conditions COX-2 is expressed at relatively high levels in discrete populations of neurons during synaptic activity and is mainly present in excitatory neurons (3). COX-2 protein or mRNA was detected in neurons as well as in the non-neuronal cells of the central nervous system (CNS) (7). These findings suggest a role for prostaglandins in CNS transmission and raise the possibility of modulation in CNS function by selective COX-2 inhibitors (8).

Prostaglandins are known to play a role in stimulation of the hypothalamic-pituitary-adrenal axis, including that by adrenergic agents (9, 10) and neurohormonal systems (11, 12). Previous studies from our laboratory showed that indomethacin, a non-selective cyclooxygenase inhibitor, administered i.c.v. altered, to different extent, the responses of the HPA axis to stimulation by adrenergic agonists that stimulate different subtypes of adrenergic receptor (11, 13).

In the present experiment we investigated the potential role of constitutive and inducible cyclooxygenase and prostaglandins synthesized by these isoenzymes in central adrenergic stimulation of the HPA axis in rats under basal conditions.

MATERIALS AND METHODS

Male Wistar rats weighing 190—220 g were used in these studies. The animals were housed in solid bottom cages with sawdust litter, 6 per cage and were fed on commercial food and water ad libitum. The animal room was maintained on a 12 h light/dark cycle. All animals were given a one-week acclimation period before the onset of experimentation. For intracerebroventricular injections, the skuls of rats were prepared one day earlier under light ether anesthesia. The rats remained in their home cages until they were scheduled for treatment. The experiments were performed in accordance with bioethical requirements and were approved by the local ethical committee.

General procedures

The experiments were performed in five groups. The rats of control groups were pretreated i.c.v. with 10 µl of saline or solvent for piroxicam or solvent for NS-398. Also piroxicam and NS-398 in doses used in experimental groups were administered i.c.v. to control rats 1 h before decapitation. Experimental groups were pretreated i.c.v. with piroxicam or NS-398. In these groups of rats the effect of subsequent i.c.v. administration of adrenergic agonists phenylephrine, clonidine, isoprenaline or clenbuterol 15 min later on ACTH and corticosterone secretion was investigated.

Induction of ACTH and corticosterone secretion

The secretion of ACTH and corticosterone was elicited by i.c.v. administration of adrenergic agonists, phenylephrine (30 µg), an α_1-adrenergic receptor agonist, clonidine (10 µg), an α_2-adrenergic receptor agonist, isoprenaline (20 µg), a non-selective β-adrenergic agonist, and clenbuterol (10 µg), a selective β_2-adrenergic agonist.

Inhibition of the constitutively expressed cyclooxygenase (COX-1) was induced by pretreatment of rats 15 min before each of adrenergic agonist with piroxicam (0.02-0.2 µg i.c.v.) and inhibition of inducible COX isoenzyme was induced by pretreatment with a highly selective COX-2 inhibitor NS-398 (0.01 and 0.1 µg i.c.v.).

Preparation of drugs

Drugs used in this study were: L-phenylephrine hydrochloride, DL-isoproterenol hydrochloride, clenbuterol hydrochloride, piroxicam (Sigma), NS-398 (Cayman Chemical Co) and clonidine (Boehringer). The doses used are expressed in terms of salts. Piroxicam was prepared for injection by sonication in 1% Tween solution, NS-398 was dissolved in ethanol and adrenergic agonists were dissolved in saline. Solutions were prepared immediately before use. The drugs or solvents were administered i.c.v. in a volume of 10 µl per rat.

ACTH and corticosterone determinations

One hour after the last injection the rats were decapitated immediately after their removal from the cage and their trunk blood samples were collected on ice in plastic conical tubes containing 200 µl of a solution of 5 mg/ml EDTA and 500 TIU of aprotinin (Sigma). Control rats were decapitated concurrently

with the experimental group. Plasma was separated by centrifugation in a refrigerated centrifuge within 30 min and frozen at -80°C until the time of assay. Plasma ACTH concentrations were measured using the double antibody ^{125}I radioimmunoassay obtained from CIS Bio International and calculated as pg/ml of plasma. The concentration of serum corticosterone was measured fluorometrically and expressed as µg per 100 ml. To avoid circadian variability, all experiments were performed between 10—11 a.m. and all decapitations between 11—12 a.m., when plasma hormones are at a relatively low levels.

Statistics

The results were calculated as a group mean ± standard error of the mean. Statistical evaluation was performed by an analysis of variance, followed by

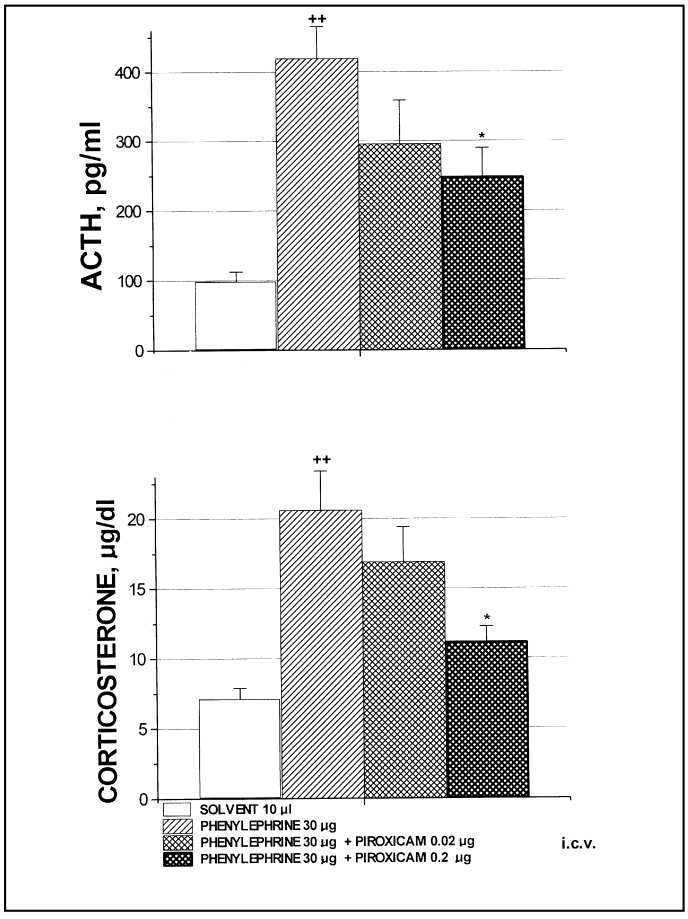

Fig. 1. Effect of piroxicam on the phenylephrine--induced ACTH and corticosterone secretion. Piroxicam was injected i.c.v. 15 min before i.c.v. phenylephrine. In Fig. 1—8 one hour after the last injection the rats were decapitated. Values represent the mean ± SEM of 6 rats. +$p < 0.05$ and ++$p < 0.01$ vs saline conrol group; *$p < 0.05$ vs. respective adrenergic agonist treated group.

individual comparisons with Duncan's test. The results were considered to be significantly different when $p < 0.05$.

RESULTS

Effect of COX-1 and COX-2 inhibitors on phenylephrine-induced ACTH and corticosterone secretion

In control groups neither the solvents nor piroxicam or NS-398 given i.c.v. alone in doses used in experimental groups elicited any marked alterations in plasma ACTH and serum corticosterone levels in comparision with i.c.v. saline treated groups.

Phenylephrine (30 µg), an α_1-adrenergic receptor agonist, administered i.c.v. to conscious rats elicited significant increase in ACTH and corticosterone

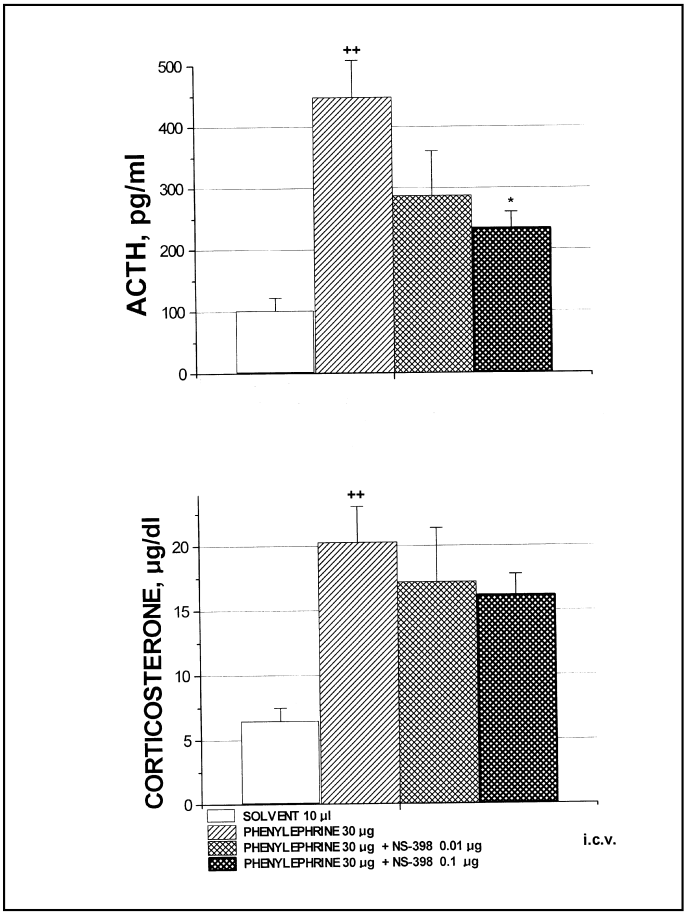

Fig. 2. Effect of the cyclooxygenase-2 inhibitor NS-398 on the phenylephrine-induced ACTH and corticosterone response. See legend to *Fig. 1.*

secretion measured 1 h later. Pretreatment with piroxicam (0.02 or 0.2 μg), a constitutive cyclooxygenase (COX-1) inhibitor, 15 min before phenylephrine dose-dependently diminished the phenylephrine-induced ACTH and corticosterone secretion. In a lower dose (0.02 μg) piroxicam markedly diminished (38.6 and 27.4%) and in a higher dose (0.2 μg) it considerably reduced (52.3 and 69.6%) the phenylephrine-evoked increase in ACTH and corticosterone secretion (*Fig. 1*). Compound NS-398, a selective inhibitor of inducible cyclooxygenase (COX-2), also dose-dependently decreased the phenylephrine-induced ACTH and corticosterone responses. Given in a lower dose (0.01 μg) this inhibitor strongly diminished (46.4 and 22.5%) and in a higher dose (0.1μg) it significantly reduced (61.5%) the phenylephrine-induced ACTH secretion and to a lesser extent (30%) decreased corticosterone secretion (*Fig. 2*).

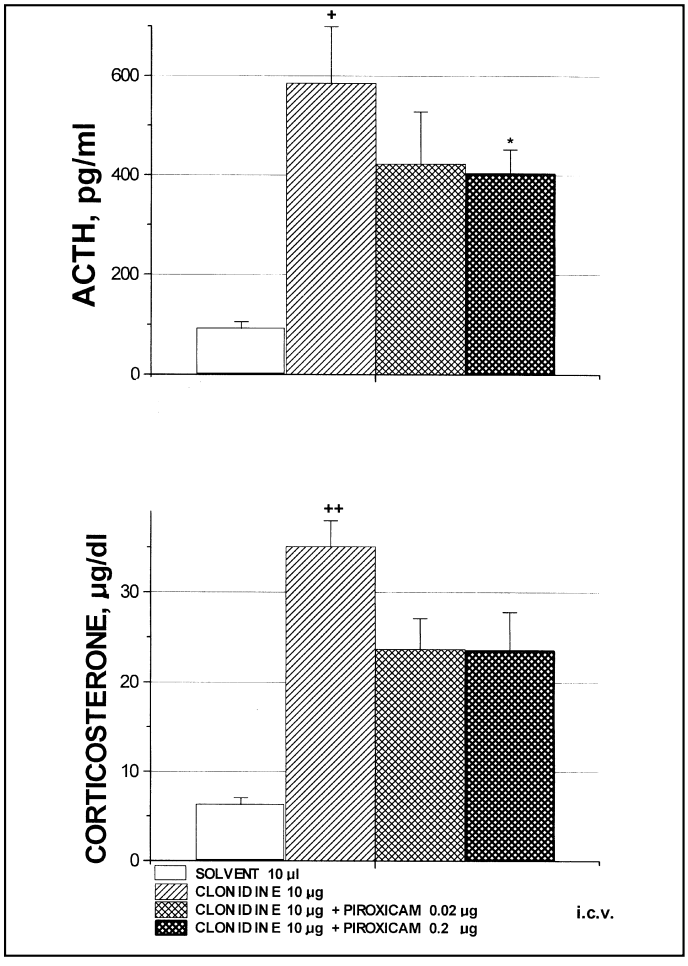

Fig. 3. Effect of piroxicam on the clonidine-elicited ACTH and corticosterone secretion. See legend do *Fig. 1.*

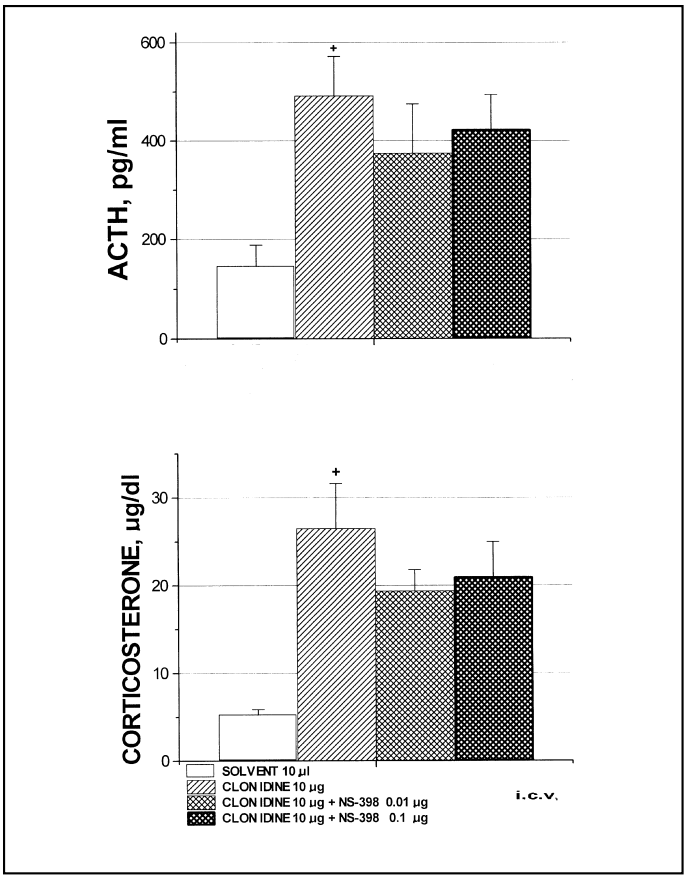

Fig. 4. Effect of NS-398 on the clonidine-induced ACTH and corticosterone response. See legend to *Fig. 1*.

Effects of COX-1 and COX-2 inhibitors on clonidine-induced ACTH and corticosterone responses

A significant increase in ACTH and corticosterone secretion induced by i.c.v. clonidine (10 µg), an α_2-adrenergic receptor agonist was markedly, though not significantly, decreased by pretratment with piroxicam (0.02 or 0.2 µg) by up to 22.2 and 33.5%, respectively (*Fig. 3*). Pretreatment with NS-398 did not substantially alter the clonidine-induced ACTH and corticosterone responses (*Fig. 4*).

Effect of COX-1 and COX-2 inhibitors on isoprenaline-induced ACTH and corticosterone responses

Piroxicam (0.02 or 0.2 µg i.c.v.) administered 15 min prior to isoprenaline (20 µg), a non-selective β-adrenergic receptor agonist, did not markedly affect

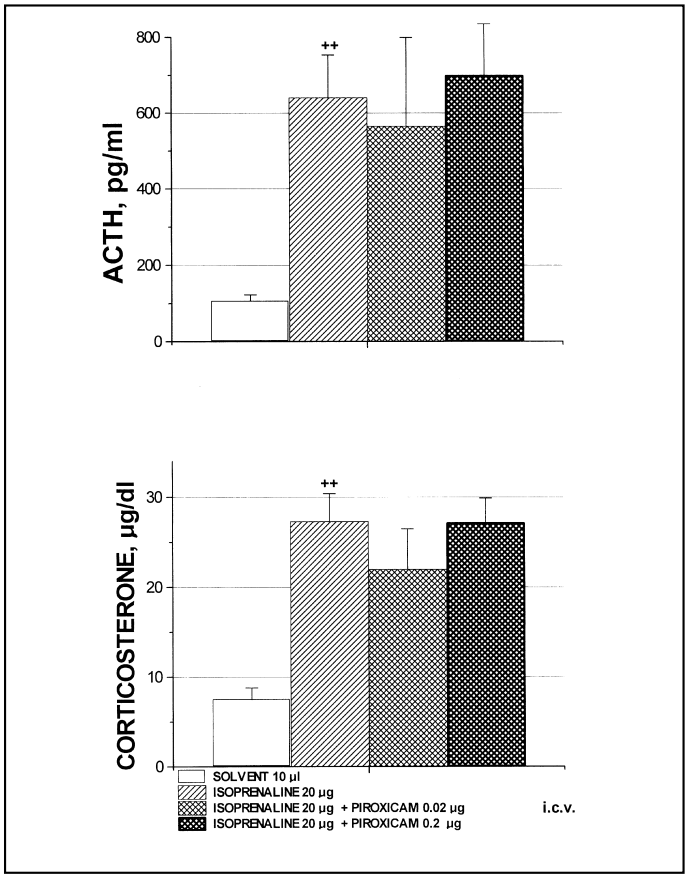

Fig. 5. Effect of piroxicam on the isoprenaline-induced ACTH and corticosterone response. See legend to *Fig. 1*.

the isoprenaline-induced ACTH and corticosterone secretion. In a lower dose (0.02 µg) piroxicam moderately decreased (14.1 and 26.8%) the isoprenaline--induced ACTH and corticosterone secretion, while in a higher dose (0.2 µg) it slightly increased (by 11.2%) the isoprenaline-evoked ACTH response and did not affect corticosterone secretion (*Fig. 5*). Likewise, compound NS-398, in both doses used (0.01 and 0.1 µg) moderately diminished (25.5 and 21.4%) the isoprenaline-induced increase in ACTH secretion, while it modestly augmented (25.2 and 18.8%) the isoprenaline-induced corticosterone secretion (*Fig. 6*).

Effect of COX-1 and COX-2 inhibitors on clenbuterol-induced ACTH and corticosterone responses

In the present experiment clenbuterol (10 µg i.c.v.), a selective β_2-adrenergic receptor agonist, induced much stronger ACTH and corticosterone secretion than the non-selective β- and α-adrenergic agonists used in this study. The

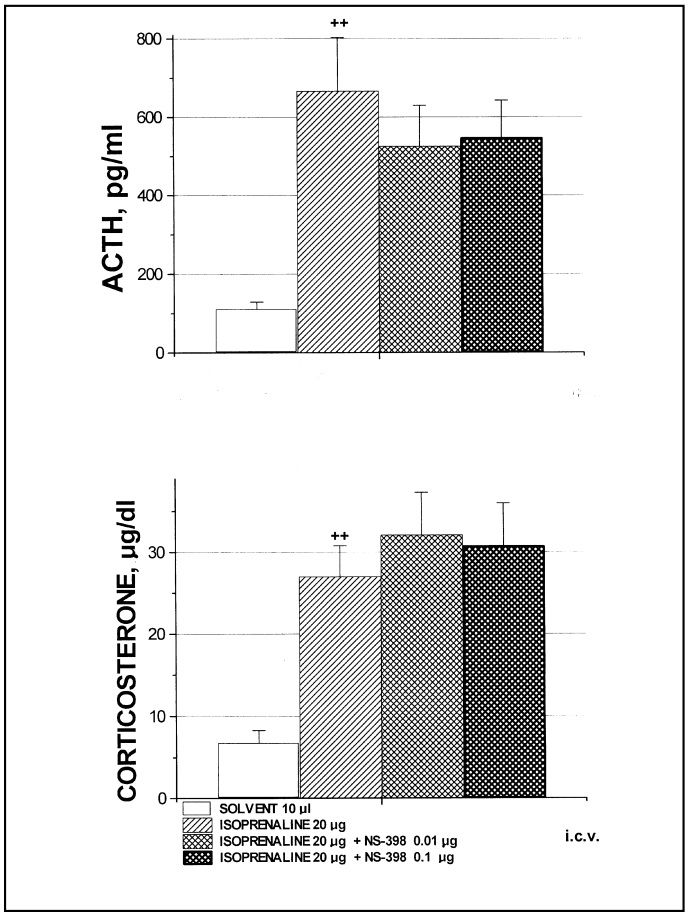

Fig. 6. Effect of NS-398 on the isoprenaline-induced ACTH and corticosterone response. See legend to *Fig. 1.*

clenbuterol-induced increases in ACTH and corticosterone secretion were significantly reduced (53.7 and 34.2%) by pretreatment with piroxicam in a larger dose (0.2 µg), whereas in a lower dose (0.02 µg) it slightly increased (15.6 and 2.8%) these responses (*Fig. 7*). The COX-2 inhibitor, NS-398 in a dose of 0.1µg markedly diminished the clenbuterol-induced ACTH (41%) and did not significantly weakened (14%) corticosterone secretion. Administered in a lower dose (0.01µg) NS-398 did not substantially alter the clenbuterol--induced ACTH and corticosterone secretion (*Fig. 8*).

DISCUSSION

In the present experiment pretreatment with the cyclooxygenase-1 inhibitor piroxicam significantly reduced the phenylephrine-induced increase in ACTH and corticosterone secretion, by 52 and 70%, respectively. Likewise NS-398, a

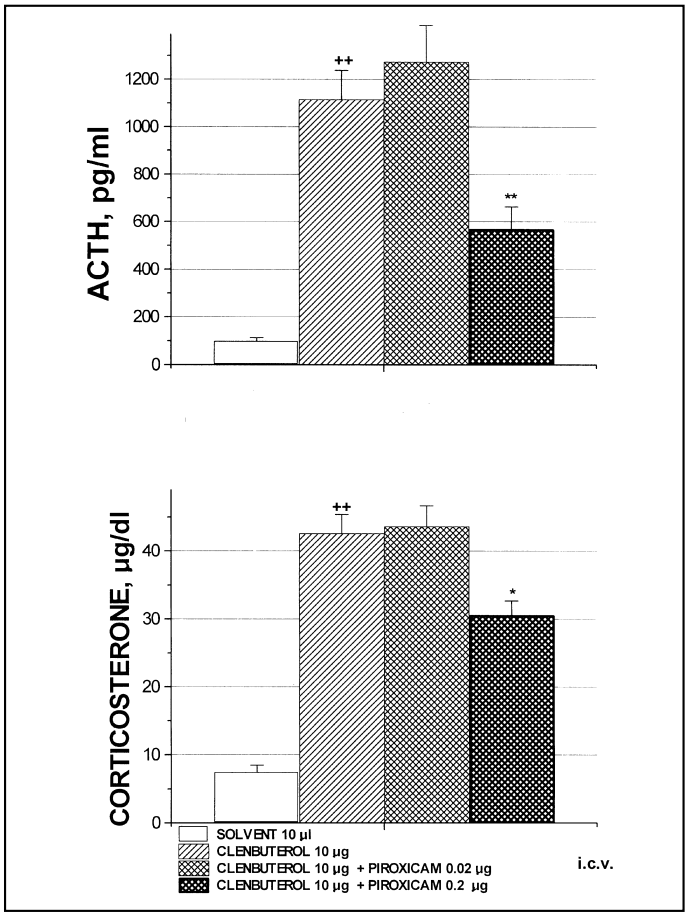

Fig. 7. Effect of piroxicam on the clenbuterol-induced ACTH and corticosterone response. See legend to *Fig. 1.*

COX-2 inhibitor, significantly and dose-dependently diminished the phenylephrine-induced ACTH response and to a lesser extent decreased corticosterone secretion. These findings indicate that in brain structures involved in activation of the HPA axis both COX-1 and COX-2 are present and prostaglandins synthesized by these isoenzymes considerably and to a similar extent mediate the HPA response to α_1-adrenergic receptor stimulation. The occupancy and activation of α_1-adrenergic receptors is known to stimulate rapid hydrolysis of membrane phospholipid to inositol triphosphate (IP_3) and intracellular calcium mobilization. The mechanism of activation of a calcium channel is related to the action of α_{1A} subtype of adrenergic receptor, whereas the $_{1B}$ receptor exerts its effect through the second messenger inositol triphosphate (14). High levels of α_{1A}- and α_{1B}-adrenergic receptor mRNA were observed in the hypothalamic paraventricular nucleus where CRH is synthesized and released during adrenergic stimulation (15, 16). Alpha$_1$-adrenergic receptors

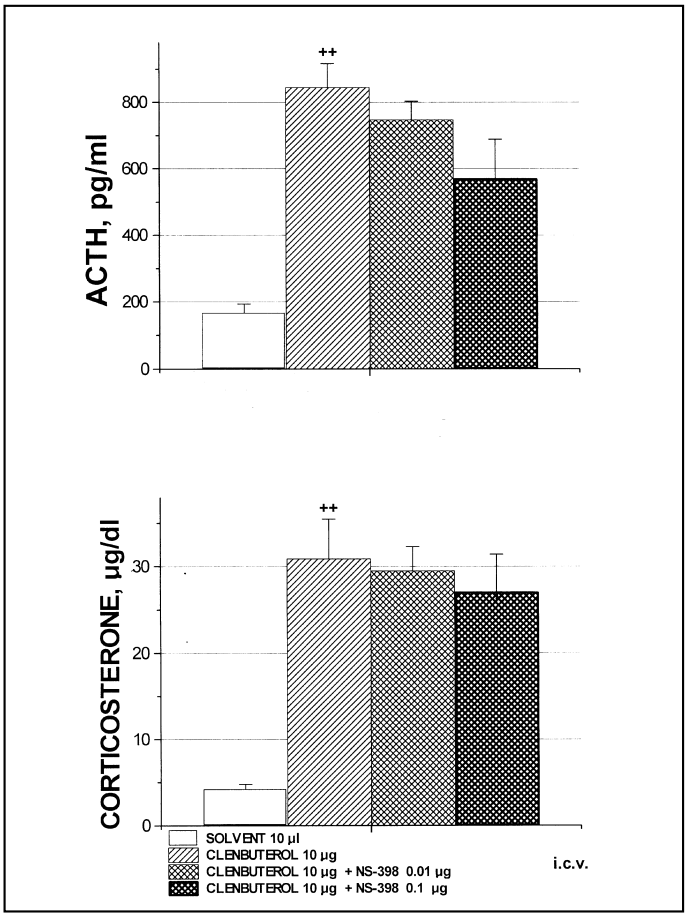

Fig. 8. Effect of NS-398 on the clenbuterol-induced ACTH and corticosterone response. See legend to *Fig. 1*.

also mediate the release of arachidonic acid induced by the activation of phospholipase A_2 (17, 18) which is the rate-limiting step for the generation of eicosanoids from membrane phospholipids in neurons.

A significant diminution by COX-1 and COX-2 inhibitors of the phenylephrine-induced ACTH and corticosterone responses in the present experiment suggests that the phenylephrine-induced arachidonic acid release results in increased prostaglandins synthesis by both COX-1 and COX-2 pathway. Moreover, a similar inhibition of ACTH secretion by piroxicam and NS-398, by 52 and 61%, suggests a roughly equal participation of COX-1 and COX-2 isoenzymes in prostaglandins synthesis stimulated via α_1-adrenergic receptors in hypothalamic structures involved in CRH release. Gradual decreases in phenylephrine-induced corticosterone secretion by piroxicam and NS-398 were, according to expectation, parallel with declines in ACTH secretion.

COX-1 inhibitor piroxicam (0.02 and 0.2 μg i.c.v.) induced a moderate decreases in the clonidine-induced ACTH (5.3 and 22.2%) and corticosterone responses (27.7 and 33.5%), which were not statistically significant in comparision with the clonidine-elicited responses. Also COX-2 blocker NS-398 (0.01 and 0.1 μg) did not result in any regular or significant alterations in the clonidine-evoked increase in ACTH and corticosterone levels. These results suggest that prostaglandins, particularly synthesized by COX-1 izoenzyme, may be moderately involved in the ACTH and corticosterone response to clonidine, an α_2-adrenergic agonist. Although activation of α_2-adrenergic receptor is coupled to the Gi and results mainly in inhibition of adenylyl cyclase, in some systems, such as smooth muscle and kidney epithelial cells, it results in stimulation of phosphoinositol-phospholipase C, thus triggering calcium entry, which in turn leads to phospholipase A_2 activation and the accumulation of arachidonic acid. Activation of α_2-, and α_1-adrenergic receptors by clonidine and phenylephrine is known to release prostaglandins from venous endothelium (19). It has not been elucidated whether i.c.v. clonidine which stimulates α_2-adrenoceptors on CRH secreting neurons in the hypothalamic paraventricular nucleus (20) also releases prostaglandins. It is also unclear, whether and to what extent, these actions of α_2-adrenoceptors can affect the function of both COX-isoenzymes during central HPA axis stimulation with clonidine. Our earlier study showed significant involvement of prostaglandins in the ACTH and corticosterone response to i.c.v. clonidine (21). Indomethacin (10 μg i.c.v.), a non-selective COX inhibitor, considerably diminished the clonidine-induced increase in plasma ACTH and serum corticosterone levels (45 and 43%) (21, 22). The indomethacin-induced inhibition was greater than that elicited by either piroxicam or NS-398, a COX-1 or COX-2 inhibitor, in the present experiment and may represent a sum of the inhibitory action on both cyclooxygenases. Our present results indicate the involvement of prostaglandins synthesized by COX-1 and COX-2 in the clonidine induced activation of the HPA axis.

Isoprenaline (20 μg i.c.v.), a non-selective β-adrenergic receptor agonist, considerably augmented ACTH and corticosterone secretion. Isoprenaline--induced ACTH and corticosterone secretion was moderately, but not significantly diminished by pretreatment with COX-1 inhibitor piroxicam (0.02 μg i.c.v.). Likewise, COX-2 inhibitor, NS-398 did not significantly diminish the isoprenaline-elicited increase in ACTH secretion and moderately increased corticosterone response. Activation of β-adrenergic receptors increases intracellular cAMP levels and the synthesis of prostanoids in various tissues, in the heart and the cultured coronary endothelial cells (23). The present results corroborate the assumption that prostaglandins synthesized by both cyclooxygenase isoenzymes via cAMP activation may be moderately involved in the isoprenaline-induced ACTH secretion under basal in vivo conditions.

Although isoprenaline has equal affinity for β_1- and β_2-adrenoceptors, its central administration induces a preferential regulation of β_2-adrenoceptors in different brain structures, including the hypothalamus (24). Therefore, significant stimulation of ACTH and corticosterone secretion by i.c.v. isoprenaline in the present experiment may result from preferential β_2-adrenoceptors activation.

Clenbuterol, a selective β_2-adrenergic receptor agonist, is far more potent than isoprenaline in stimulation of the HPA axis. The clenbuterol-induced considerable rise in ACTH and corticosterone secretion, was significantly reduced by COX-1 inhibitor, piroxicam (0.2 μg i.c.v.) by 54 and 34%, respectively. Also COX-2 inhibitor NS-398 (0.1 μg i.c.v.) markedly, though less potently, decreased this secretion, (41 and 14%). This observation suggests that prostaglandins synthesized by both cyclooxygenase isoenzymes, COX-1 and COX-2, very markedly mediate ACTH and corticosterone secretion stimulated centrally by clenbuterol, a selective β_2-adrenergic receptor agonist.

We have shown that in the adrenergic-induced ACTH and corticosterone secretion endogenous nitric oxide (NO) is significantly involved (25). Inhibition of central neuronal nitric oxide synthase (NOS) by its antagonist N^G-nitrol-L-arginine methyl ester considerably reduced the ACTH and corticosterone responses to α_1- and α_2-adrenergic receptors stimulation while the stimulation induced by β-adrenergic receptors was only moderately affected. These results agree with the effects of COX blockers on adrenergic-induced HPA responses in the present experiment and suggest that inducible COX and iNOS can be coexpressed in hypothalamic structures involved in the stimulation of HPA axis by i.c.v. administered adrenergic receptor agonist. Nitric oxide is known as a mediator of increased COX-2 expression in different cell populations, rheumatoid synovial cells (26), rat skin during neurogenic hyperaemia (27) and in increased renal cortical COX-2 expression system during regulation of arterioral tone (28). However, the exact interaction between the COX and NO systems in the activation of HPA response by central stimulation of adrenergic receptors is not exactly known.

In summary, the present study shows that in conscious rats under basal conditions inducible cyclooxygenase activity is present in brain structures involved in the regulation of HPA axis. Both COX-1 and COX-2 isoenzymes participate to a roughly equal extent in central stimulation of the HPA axis by adrenergic receptor agonists. The most significant involvement of both cyclooxygenase isoenzymes was observed during α_1- and β_2-adrenergic receptors-induced stimulation of the HPA axis. The involvement of these cyclooxygenases was moderately expressed in the α_2- and β-adrenergic receptors-induced HPA responses.

Acknowledgements: This study was supported by the State Scientific Research Committee (KBN), grant No. 4 P05A 127 18.

REFERENCES

1. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. *Annu Rev Pharmacol Toxicol* 1998; 38: 97—120.
2. Vane JR, Botting RM. The fight against rheumatism: from willow bark to COX-1 sparing drugs. *J Physiol Pharmacol* 2000; 51, Part I: 573—586.
3. Yamagata K, Andreasson KI, Kaufmann WE, Barnes CA, Worley PF. Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. *Neuron* 1993; 11: 371—386.
4. Katori M, Majima M. Cyclooxygenase-2: its rich diversity of roles and possible application of its selective inhibitors. *Inflamm Res* 2000; 49: 367—392.
5. O'Banion MK, Olschowka JA. Localization and distribution of cyclooxygenase-2 in brain tissue by immunohistochemistry. Methods in Molecular Biology, vol 120, Eicosanoid Protocols ed by: E.A. Lianos, Humana press Inc., Totowa, New York.
6. Beiche F, Scheuerer S, Brune K, Geisslinger G, Goppelt-Struebe M. Up-regulation of cyclooxygenase-2 mRNA in the rat spinal cord following peripheral inflammation. *FEBS Letters* 1996; 390: 165—169.
7. Lacroix S, Rivest S. Effect of acute systemic inflammatory response and cytokines on the transcription of the genes encoding cyclooxygenase enzymes (COX-1 and COX-2) in the rat brain. *J Neurochem* 1998; 70: 452—466.
8. Schaad NC, Magistretti PJ, Schorderet M. Prostanoids and their role in cell-cell interactions in the central nervous system. *Neurochem Int* 1991; 18: 303—322.
9. Watanabe T, Morimoto A, Morimoto K, Nakamori T, Murakami N. ACTH release induced in rats by noradrenaline is mediated by prostaglandin E_2. *J Physiol* 1991; 443: 431—439.
10. Watanabe T, Morimoto A, Sakata Y, Murakami N. ACTH response induced by interleukin-1 is mediated by CRF secretion stimulated by hypothalamic PGE. *Experientia* 1990; 46: 481—484.
11. Bugajski J. Role of prostaglandins in the stimulatory action of the hypothalamic-pituitary--adrenal axis by adrenergic and neurohormone systems. *J Physiol Pharmacol* 1996; 47: 559—575.
12. Bugajski J. Social stress adapts signaling pathways involved in stimulation of the hypothalamic-pituitary-adrenal axis. *J Physiol Pharmacol* 1999; 50: 367—379.
13. Bugajski J, Gądek-Michalska A, Borycz J, Głód R, Bugajski AJ. Effect of indomethacin on the pituitary-adrenocortical response to adrenergic stimulation. *Life Sci* 1996; 59: 1157—1164.
14. Bylund DB. Subtypes of α_1- and α_2-adrenergic receptors. *The FASEB Journal* 1992; 6: 832—839.
15. Day HEW, Campeau S, Watson SJ Jr, Akil H. Distribution of α_{1A}-, α_{1B}- and α_{1D}-adrenergic receptor mRNA in the rat brain and spinal cord. *J Chem Neuroanatomy* 1997; 13: 115—139.
16. Acosta-Martinez M, Fiber JM, Brown RD, Etgen AM. Localization of α_{1B} -adrenergic receptor in female rat brain regions involved in stress and neuroendocrine function. *Neurochem International* 1999; 35: 383—391.
17. Axelrod J. Receptor-mediated activation of phospholipase A_2 and arachidonic acid release in signal transduction. *Biochem Soc Transductions* 1990; 18: 503—507.
18. Edwards L, Ernsberger P. Assay of arachidonic acid release coupled to α_1- and α_2 –adrenergic receptors. Methods in Molecular Biology, vol. 126: Adrenergic Receptor Protocols ed by: CA Machida, Humana press Inc., Totowa, New York.
19. Callow ID, Campisi P, Lambert ML, Feng Q, Arnold JM. Enhanced in vivo alpha1- and alpha2-adrenoceptor-mediated venoconstriction with indomethacin in humans. *Am J Physiol* 1998 Sep; 275 (3Pt2): H837—843.

20. Roland CR, Bhakthavatsalam P, Leibovitz SF. Interaction between corticosterone and alpha-2-noradrenergic system of the paraventricular nucleus in relation to feeding behavior. *Neuroendocrinology* 1986; 42: 296—305.
21. Bugajski J, Gądek-Michalska A, Borycz J, Głód R, Ołowska A. The role of prostaglandins and the hypothalamic and hippocampal histamine in the clonidine-induced pituitary--adrenocortical response. *J Physiol Pharmacol* 1996; 47: 487—495.
22. Głód R, Gądek-Michalska A, Bugajski J. The influence of indomethacin on the ACTH secretion induced by central stimulation of adrenergic receptors. *J Physiol Pharmacol* 2000; 51: 347—357.
23. Ruan Y, Kan H, Malik KU. Beta adrenergic receptor stimulated prostacyclin synthesis in rabbit coronary endothelial cells is mediated by selective activation of phospholipase D: inhibition by adenosine 3'5'-cyclic monophosphate. *J Pharmacol Exp Therapeut* 1997; 281: 1038—1046.
24. Gambarana C, Ordway GA, Hauptmann M, Tejani-Butt S, Frazer A. Central administration of 1-isoproterenol in vivo induces a preferential regulation of β_2 adrenoceptors in the central nervous system of the rat. *Brain Res* 1991; 555: 141—148.
25. Bugajski J, Gądek-Michalska A, Głód R, Borycz J, Bugajski AJ. Blockade of nitric oxide formation impairs adrenergic-induced ACTH and corticosterone secretion. *J Physiol Pharmacol* 1999; 50: 327—335.
26. Honda S, Migita K, Hirai Y, Ueki Y, Yamasaki S, Urayama S, Kawabe Y, Fukuda T, Kawakami A, Kamachi M, Kita M, Ida H, Aoyagi T, Eguchi K. Induction of COX-2 expression by nitric oxide in rheumatoid synovial cells. *Biochem Biophys Res Commun* 2000; 268: 928—931.
27. Holzer P, Jocic M, Peskar BA. Mediation by prostaglandins of the nitric oxide-induced neurogenic vasodilatation in rat skin. *Brit J Pharmacol* 1995; 116: 2365—2370.
28. Cheng H-F, Wang J-L, Zhang M-Z, McKanna JA, Harris RC. Nitric oxide regulates renal cortical cyclooxygenase-2 expression. *Am J Physiol Renal Physiol* 2000; 278: F122—F129.

R e c e i v e d: July 29, 2001
A c c e p t e d: October 18, 2001

Author's address: Prof. J. Bugajski, Department of Physiology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Kraków, Poland.