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Biochemical Pharmacology, Vol. 32, No. 4, pp. 729–732, 1983.
Printed in Great Britain.

0006-2952/83/040729-04 $03.00/0

Norepinephrine and prostaglandin biosynthesis by iris smooth muscle and iris microsomes

(*Received* 15 *April* 1982; *accepted* 23 *August* 1982)

Catecholamines have been reported to stimulate prostaglandin (PG) biosynthesis in a variety of tissues including spleen [1, 2], heart [3], phrenic diaphragm [4], kidney [5], brain [6], rabbit mesenteric blood vessels [7], rabbit iris smooth muscle [8–10], and cultured cells [11, 12]. The mechanism underlying the stimulatory effect of these amines on PG synthesis is poorly understood. It has been suggested that: (a) they act as cofactors for the cyclooxygenation of arachidonic acid (AA), as shown by their stimulatory effects on PG synthetase activity [13]; and (b) they stimulate PG synthesis through receptor-mediated mechanisms; thus, treatment with the α-adrenergic receptor blocking agent phenoxybenzamine inhibited the appearance of PGs from dog spleen [1] and rabbit kidney [5]. Furthermore, PG synthesis in cell cultures from dog kidney cells [11] and from rabbit splenic pulpa [12] is mediated through α-adrenoreceptors.

In a previous communication from this laboratory [9], we reported that, in rabbit iris smooth muscle, norepinephrine (NE) stimulated PG synthesis in a dose-dependent manner. The NE stimulation of PG synthesis was blocked by indomethacin [10]. To throw more light on the mechanism of NE stimulation of PG release in this tissue, we have compared the effects of NE and other catecholamines on conversion of [1-^{14}C]AA into PGs by iris muscle and iris microsomes.

Materials and methods

[1-^{14}C]AA (sp. act. 56.5 mCi/mmole) was purchased from the Amersham Corp., Arlington Heights, IL; NE and other catecholamines were purchased from the Sigma Chemical Co., St. Louis, MO.

In general, two rabbit irides from pairs were incubated (of the pair, one was used as control) in 1 ml of isoosmotic medium that contained 0.25 μCi of [1-^{14}C]AA (sp. act. 56.5 mCi/mmole) bound to 0.1 mg albumin at 37° for 1 hr. Catecholamines and other agents were added as indicated. At the end of incubation the medium was analyzed for PGs.

Microsomes were prepared from iris muscle as previously described [14]. Microsomes, equivalent to 0.6 mg protein, were incubated in 1 ml of 0.1 M phosphate buffer, pH 7.8, containing 0.25 μCi AA, in the presence and absence of the drug as indicated, at 37° for 1 hr. At the end of incubation the PGs were extracted and analyzed.

The medium was analyzed for prostaglandins. It was acidified with 10% formic acid to pH 3.5 and extracted three times with 3 ml of ethylacetate. The solvent was evaporated under nitrogen. The residue was dissolved in chloroform–methanol (2:1), spotted on Whatman precoated silica gel LK6DF plates, and developed in a solvent system [15] of ethylether–methanol–acetic acid (90:1:2, by vol.). After visualization of the PG standards by exposure to iodine vapor, the radioactive PG spots were located with autoradiography, and their radioactive contents were measured by counting in a Beckman liquid scintillation counter. In the present study, we have analyzed for $PGF_{2\alpha}$ and PGE_2. Data are reported as ^{14}C-radioactivity (cpm) of AA converted into PGs/two rabbit irides, or as ^{14}C-radioactivity converted into PGs/mg of iris microsomal proteins.

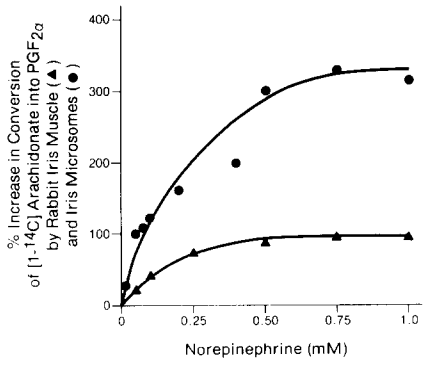

Fig. 1. Effect of NE concentration on conversion of [1-^{14}C]AA into $PGF_{2\alpha}$ by rabbit whole iris (▲) and iris microsomes (●). In these experiments, two irides from pairs were incubated in 1 ml of an isoosmotic medium that contained 0.25 μCi of [1-^{14}C]AA (sp. act. 56.9 mCi/mmole) bound to 0.1 mg albumin in the absence and presence of various concentrations of NE for 1 hr at 37°. At the end of incubation, the medium was analyzed for PGs as described under Materials and methods. The data presented for whole iris are means of two separate experiments, and each experiment was carried out in duplicate, with variability less than 7%. Assay conditions for conversion of [1-^{14}C]AA into PGs by iris microsomes are given under Materials and methods; each point represents the mean of three experiments. S.E.M. were less than 5%.

All experiments were carried out in duplicates or triplicates.

Results and discussion

Effect of NE concentration on PG synthesis in rabbit iris and iris microsomes. Comparative studies on the effects of various concentrations of NE on PG synthesis from [1-^{14}C]AA in rabbit iris muscle and iris microsomes showed that the catecholamine-induced release of $PGF_{2\alpha}$ by microsomes was considerably higher than that of the whole iris at all concentrations studied (Fig. 1). Thus, at 0.5 mM NE, a concentration at which the catecholamine-induced release of PGs levelled off, $PGF_{2\alpha}$ synthesis in muscle increased by 80% and that of microsomes increased by 300%. Similarly, at lower concentrations of NE (0.1 mM), $PGF_{2\alpha}$ synthesis by muscle and microsomes increased by 40 and 120% respectively. It can be concluded that, in the rabbit iris, NE stimulation of PG synthesis by the microsomes is three times as high as that of the muscle. NE also increases the basal release of PGE_2 (measured by means of radioimmunoassay) in a dose-dependent manner [16]. Similar data have been obtained with the bovine iris (data not shown).

Effect of NE on PG synthesis by rabbit iridial processes and scraped irides. The whole iris (iris-ciliary body), which we employed in the present study, consists of iridial processes and three smooth muscles, namely dilator, sphincter and ciliary. Thus, it was of interest to determine the effect of NE on PG synthesis in iridial processes and scraped irides. As can be seen from Table 1, comparable stimulatory effects of NE on PG synthesis were found in both tissues. These data show that both iridial processes and smooth muscle have the capacity to synthesize PGs and that this synthesis is stimulated by NE in both tissues.

Effect of catecholamines on PG synthesis by rabbit iris and iris microsomes. NE, epinephrine, isoproterenol and normetanephrine in 0.1 mM concentrations increased $PGF_{2\alpha}$ synthesis in the whole iris and iris microsomes by 57–79 and 156–197% respectively (Table 2). Phenylephrine, an α-adrenergic agonist, increased $PGF_{2\alpha}$ synthesis in iris and iris microsomes by only 29 and 57% respectively. In contrast, the deaminated metabolite of NE, 3-methoxy-4-hydroxy-mandelic acid, had little effect on PG synthesis by the iris and iris microsomes. This indicates that the aliphatic polar side-chain ethylamine in catecholamines is essential for the catecholamine-stimulated PG biosynthesis by the iris. The important role played by the ethylamine side-chain of catecholamines in PG synthesis was further supported by the finding that catechol inhibited PG synthesis by the iris and iris microsomes. Inhibition of PG synthesis by catechol is dose-dependent [16].

Is the NE-stimulated PG synthesis mediated via α-adrenoreceptors? To answer this question, we investigated the effect of phentolamine, an α-adrenergic antagonist, on NE-stimulated PG synthesis in rabbit whole iris and iris microsomes. Phentolamine reduced the NE-stimulated $PGF_{2\alpha}$ synthesis in the whole iris by 52% and in iris microsomes by 22% (Table 3). Phentolamine alone had little effect on PG synthesis. Phentolamine also blocks the NE-stimulated basal release of PGE_2 by the whole iris [16].

In conclusion, the data presented in this paper demonstrated that NE stimulated the conversion of [1-^{14}C]AA to PGs by rabbit irides, iridial processes, scraped irides and iris microsomes. NE also stimulated PG synthesis by bovine irides (data not shown). Both the catechol nucleus and the ethylamine side-chain were required for maximum activation of PG synthesis by catecholamines. Thus, the concept that catechols act as reducing agents to facilitate the cyclooxygenase pathway needs to be reexamined. The finding that phentolamine did block NE-induced PG release suggests the involvement of α-adrenoreceptors. NE has been reported to increase the release of Ca^{2+} from intracellular sites in the iris [17]. Thus, activation of adrenoreceptors by catecholamines could bring about a release of AA from membrane phospholipids, mediated via Ca^{2+} activation of phospholipase A_2, and consequently an increase in PG biosynthesis. There is no conclusive experimental evidence that shows which phospholipid is the

Table 1. Effect of NE on conversion of [1-^{14}C]AA to PGs by rabbit iridial processes and scraped irides*

Tissue	NE (mM)	$PGF_{2\alpha}$ (cpm)	% of control	PGE_2 (cpm)	% of control
Iridial processes†		10,819	100	9,011	100
	0.1	17,556	162	11,653	129
	0.5	33,125	306	20,877	232
Scraped irides‡		15,723	100	8,407	100
	0.1	29,869	190	14,265	170
	0.5	36,554	232	22,242	265

* Conditions for incubation were the same as given in the legend of Fig. 1. Data given are averages of two separate experiments.

† Processes from eight irides were incubated in each tube.

‡ Four scraped irides were incubated in each tube.

Table 2. Effects of catecholamines on conversion of [1-^{14}C]AA to PGs by rabbit iris-ciliary body and iris-ciliary body microsomes*

Additions (0.1 mM)	Effect on PG synthesis (% of control)		
	Iris		Microsomes
	$PGF_{2\alpha}$	PGE_2	$PGF_{2\alpha}$
Norepinephrine	157 ± 7	141 ± 9	297 ± 6
Epinephrine	159 ± 10	145 ± 6	256 ± 5
Phenylephrine	129 ± 9	131 ± 11	157 ± 8
Isoproterenol	157 ± 6	163 ± 8	285 ± 7
Noremetanephrine	179 ± 9	168 ± 5	294 ± 11
3-Methoxy-4-hydroxy mandelic acid	125 ± 5	127 ± 7	110 ± 4
Catechol	18 ± 3	5 ± 2	50 ± 6

* Conditions of incubation were the same as described in the legend of Fig. 1. Results are means ± S.E.M. of three different experiments, and each experiment was run in triplicate.

Table 3. Effect of phentolamine on NE-stimulated formation of PGs by rabbit whole iris and iris microsomes*

Additions	Radioactivity in PGS (% of control)			
	Whole iris		Iris microsomes	
	$PGF_{2\alpha}$	PGE_2	$PGF_{2\alpha}$	PGE_2
0.1 mM NE	160 ± 17	166 ± 18	210 ± 12	197 ± 7
0.05 mM Phentolamine	101 ± 8	103 ± 8	103 ± 3	115 ± 11
0.1 mM NE + 0.05 mM phentolamine	129 ± 9	121 ± 7	186 ± 11	179 ± 2

* Conditions of incubation were the same as described in the legend of Fig. 1. Results are means ± S.E.M. of three experiments, and each experiment was run in triplicate.

source for AA in PG synthesis. Furthermore, the precise lipase(s) activated upon hormonal stimulation of the cell remains to be established. Thus, catecholamines appear to stimulate PG synthesis in two ways: (a) they are taken up by the tissue where they act as cofactors for the cyclo-oxygenation of AA. ([^{14}C]NE is taken up rapidly by the iris [16]) and (b) they stimulate PG synthesis through adrenoreceptor-mediated mechanisms.

Stimulation of PG synthesis by NE may be of importance in normal or pathological situations. In the eye, the increased release of catecholamines from neurons may serve as a stimulus for increased AA release and subsequent PG synthesis. Furthermore, catecholamines and adrenergic drugs are routinely employed therapeutically to lower intraocular pressure in the eye; a catecholamine-induced increase in PG synthesis may play a role in mediating the effects of these therapeutic agents.

Acknowledgements—This work was supported by NIH Grant EY-04387 from the National Eye Institute. This is contribution No. 0711 from the Department of Cell and Molecular Biology, Medical College of Georgia.

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Biochemical Pharmacology, Vol. 32, No. 4, pp. 732–735, 1983.
Printed in Great Britain.

0006-2952/83/040732-04 $03.00/0
Pergamon Press Ltd.

Effects of acute and chronic desmethylimipramine on levels of cyclic AMP *in vivo**

(*Received* 15 *April* 1982; *accepted* 23 *August* 1982)

Although the clinically relevant mechanism of action of tricyclic antidepressants including desmethylimipramine (DMI) is not certain, DMI clearly affects various aspects of noradrenergic synaptic function. DMI has been shown to decrease the reuptake of norepinephrine acutely [1, 2], and chronic DMI administration has been shown to desensitize alpha-adrenergic presynaptic receptors [3, 4], decrease firing rates of locus coeruleus neurons [5], increase 3-methoxy-4-hydroxyphenylglycol (MHPG) levels [6, 7], and decrease the sensitivity of beta-adrenergic receptors [8, 9]. Since the time required for therapeutic efficacy of DMI and the development of subsensitive beta-receptors share a similar time course, it has been suggested that the change in beta-receptor sensitivity is important in the antidepressant mechanism of action of DMI.

The beta-adrenergic receptor in the CNS appears to be linked to adenylate cyclase, and the pharmacological effects of beta-adrenergic stimulation by catecholamines can be assessed by measuring cyclic AMP response [10, 11]. *In vitro* studies have shown diminished cyclic AMP response to exogenous noradrenergic stimulation in tissue prepared from rats chronically treated with DMI [12–16]. Pineal cyclic AMP response to isoproterenol has been shown to be decreased *in vivo* following chronic administration of DMI [17].

Using a high-power microwave system [18, 19] to rapidly inactivate adenylate cyclase and phosphodiesterase, our laboratory has been investigating the cyclic AMP and cyclic GMP responses to various neurotransmitter agonists in discrete rat brain regions *in vivo* [20–22].

In this report, we describe the effects of a single acute dose of DMI on cyclic AMP levels in twenty-one brain areas as well as the effects of chronic DMI treatment on basal levels of cyclic AMP in rat brain regions. Chronically treated groups were also challenged with a beta-adrenergic agonist (isoproterenol) to assess beta-noradrenergic receptor sensitivity. DMI has been reported to block muscarinic receptors *in vitro* [23, 24], and chronic treatment might be expected to result in supersensitive muscarinic receptors. Since we have shown a significant cyclic AMP response *in vivo* to cholinergic agonists [22], DMI chronically treated rats were also challenged with a muscarinic cholinergic agonist (oxotremorine).

Methods

Animals. All experiments were performed with Wistar derived random bred male rats from the Walter Reed colony (300 ± 20 g).† The rats were housed in individual cages with food and water freely available. Lights were on from 6:00 a.m. to 6:00 p.m.

Drugs. Desmethylimipramine hydrochloride (USV Pharmaceutical Corp., Tuckahoe, NY), methylatropine nitrate, oxotremorine sesquifumarate, and DL-isoproterenol hydrochloride (Sigma Chemical Co., St. Louis, MO) were dissolved in saline immediately prior to use. Doses are expressed as the base.

Experiment 1: Effect of acute DMI on cyclic AMP. Rats were alternately injected with DMI (30 mg/kg) i.p. or an equivalent volume of saline. Thirty minutes following injection, at which time brain levels of DMI have been reported to be maximal [25], rats were killed by high power microwave irradiation.

Experiment 2: Effect of chronic DMI on cyclic AMP. Rats were injected daily with DMI (10 mg/kg) or saline for 6 weeks. Twenty hours after the last injection, groups of saline and DMI-pretreated rats were given an i.p. injection of saline, isoproterenol (10 mg/kg) or methylatropine nitrate (0.5 mg/kg). Saline and isoproterenol-treated rats were killed 10 min following injection. The methylatropine-pretreated (to prevent excess peripheral cholinergic stimulation) groups were injected with oxotremorine (2 mg/kg) 30 min after the methylatropine pretreatment and then killed 10 min later.

Sacrifice and tissue preparation. Animals were killed by a 5-sec exposure to high intensity microwave irradiation at 2450 MHz using 2.5 kW forward power [26–28]. Since brief immobilization is required during the sacrifice procedure, animals were placed in a plastic cylinder which was then inserted into the wave guide such that the longitudinal axis of the body was perpendicular to the microwave E field. After sacrifice by microwave irradiation, the rats were decapitated. The heads were briefly cooled on dry ice, the desired brain regions were dissected and weighed, and then they were sonicated in 50 mM sodium acetate buffer, pH 6.2. The sonicates were centrifuged at 12,000 *g* at 4° and the supernatant fractions were stored at −70° until assayed for cyclic AMP and cyclic GMP.

* This material has been reviewed by the Walter Reed Army Institute of Research, and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

† In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.