EFFECT OF INDOMETHACIN ON CYCLIC NUCLEOTIDE METABOLISM AND VASCULAR REACTIVITY IN RATS

N. V. Speranskaya, V. P. Kulagina, and R. I. Sokolova

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Some of the effects of prostaglandins (PG) have been shown to be realized through cyclic nucleotides (CN). In experiments on isolated segments of blood vessels, $PGF_{2\alpha}$ caused constriction and raised the cyclic GMP level, whereas isoproterenol and PGE_2 led to vasodilatation and elevation of the cyclic AMP level [7]. In these experiments PG and CN were shown to participate in the regulation of vascular tone. However, the question of the character of relations between PG and CN and their effect on vascular contractility and other tissue reactions are still being vigorously debated. Evidence of a connection between

TABLE 1. Content of Cyclic Nucleotides (in pmoles/g wet weight of tissue) in Aortic Wall of Rats

Series of experiments	Duration of experiment, weeks	Cyclic AMP	Cyclic GMP	Cyclic AMP/ cycle GMP
I) Intact (n = 10)		224±9,5	23±1,9	10,1±1,5
II) 5 mg/kg indomethacin (n = 5)	1	111±7,4*	11±2,2*	9,6±2,2
III) 2 mg/kg indomethacin (n = 5)	2	173±14,1*	33,6±4,20	5,9±1,1*
Ia) Intact (n = 6)		146±14,0	16±2,1	10,2±2,10
IV) 1% NaCl to drink (n = 6)	6	196±14,8†	10±1,7	19,4±4,0†
V) 1% NaCl to drink + 2 mg/kg indomethacin (n = 6)	6	164±19,4	27,3±2,25‡	6,1±0,86‡

*Difference significant compared with experiments of series I.
†, ‡) The same, compared with experiments of series IV and V, respectively.

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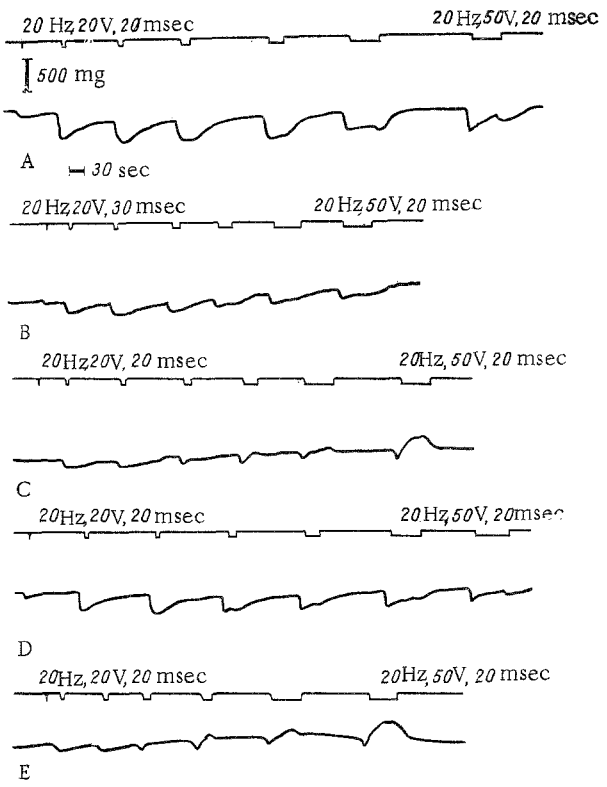

Fig. 1. Effect of indomethacin on mechanical responses of segments of abdominal aorta of rats to electrical stimulation. A) Control; B) 5 mg/kg indomethacin for 1 week; C) 2 mg/kg indomethacin for 2 weeks; D) salt loading for 6 weeks; E) 2 mg/kg indomethacin + salt loading for 6 weeks. Legend, from top to bottom: marker of stimulation, mechanogram of vascular segment.

PG and CN is given by the fact that indomethacin, an inhibitor of PG synthesis, abolishes the sodium-excretory effect of theophylline, an inhibitor of phosphodiesterases of CN [10]. However, there is information that stimulation of PG synthesis by methylxanthines is not accompanied by changes in CN phosphodiesterase activity [5].

The object of the present investigation was to assess the role of CN in the regulation of vascular reactivity in animals receiving indomethacin in chronic experiments.

EXPERIMENTAL METHOD

The following series of rats were used: I) intact (two series with ten rats in each), II) receiving large doses of indomethacin (5 mg/kg) for 2 weeks (five animals), III) receiving small doses of indomethacin (2 mg/kg) for 2 weeks (10 animals), IV) ten control rats receiving 1% NaCl to drink for 6 weeks, V) 12 rats receiving 1% NaCl and also indomethacin (2 mg/kg) daily for 6 weeks.

The CN concentration in the tissues was measured by a radioimmunologic method, using kits from the Radiochemical Centre, Amersham (England). Adenylate cyclase activity in the aorta was judged from the increase in cyclic AMP after incubation of the enzyme at 37°C in a system containing 50 mM Tris-HCl, 1 mM EDTA, 5 mM theophylline, 5 mM $MgCl_2$, 10 mM KCl, 1 mM dithiothreitol, 1 mM ATP, and an ATP-regenerating system. Activity of cyclic AMP-dependent phosphodiesterase (PDE) was determined in a partially purified tissue preparation by a radioisotope method, including thin-layer chromatography on Silufol to separate the nucleotides [4].

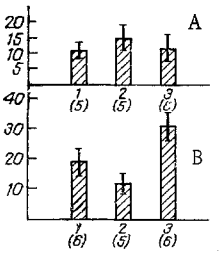

Fig. 2. AC and cyclic AMP-PDE activity in rat aorta. A) Adenylate cyclase activity in aorta; B) cyclic AMP-PDE activity in wall of aorta. Abscissa: 1) intact animals, 2) rats loaded with salt for 6 weeks, 3) rats receiving salt and indomethacin (2 mg/kg) for 6 weeks. Number of animals in each series of experiments shown in parentheses. Ordinate: A) cyclic AMP concentration (in pmoles/mg protein/min); B) AMP concentration (in nmoles/mg protein/min).

The state of vascular reactivity was assessed from reactions of segments of the abdominal aorta of rats to electrical stimulation. The preparations consisted of rings 2 mm in diameter, kept in salt solution [1]. Mechanical responses of the vascular segments to stimulation were recorded for 1, 5, 10, 20, 40, and 60 sec, with constant parameters of 20 Hz, 20 V, and 20 msec, under isometric conditions by means of a type 6 MKhlS mechanotron.

EXPERIMENTAL RESULTS

The content of cyclic AMP and cyclic GMP in the aorta of rats receiving massive doses of indomethacin was significantly reduced; the cyclic AMP/cyclic GMP ratio was not significantly changed (Table 1). Vascular responses to electrical stimulation were entirely dilating in these rats, just as in intact animals, although the degree of dilatation was rather less (Fig. 1, A and B).

In rats receiving small doses of indomethacin for 2 weeks the cyclic AMP concentration showed a tendency to fall, but the cyclic GMP concentration showed a tendency to rise. The cyclic AMP/cyclic GMP ratio was significantly lower than initially. The response of the rats of this group to electrical stimulation differed significantly from that of intact animals: dilatation was weaker still, and in response to stimulation for 60 sec vasoconstriction occurred (Fig. 1C).

In a previous study [3] the writers showed that chronic administration of small doses of indomethacin accompanied by increased NaCl intake caused the arterial blood pressure to rise to 120-160 mm Hg in 50-70% of rats. Following isolated administration of indomethacin or NaCl, hypertension was observed in only 10-20% of animals.

The cyclic nucleotide concentration in the aorta of intact rats in the experiments of series I and II differed significantly. This may be attributed to seasonal changes, different diets given in the animal house, or certain variations in fixation of the tissues. In each experiment an "internal" control was therefore used.

In animals receiving an excess of NaCl the cyclic AMP content in the aorta was significantly increased but the cyclic GMP concentration showed a tendency to fall. The cyclic AMP/cyclic GMP ratio was increased to twice its value for intact animals. This increase in the contribution of cyclic AMP to cyclic nucleotide metabolism was evidently adaptive in character and aimed at increasing exretion of sodium and water [10]. The contractility

of the aorta in rats receiving an excess of NaCl did not differ appreciably from that in intact animals (Fig. 1D).

However, after combined administration of salt solution and indomethacin (2 mg/kg) the response of the blood vessels was significantly changed: Marked constrictor reactions appeared in response not only to prolonged and strong stimulation, but also to stimulation of shorter duration — 40 sec (Fig. 1E). The cyclic GMP content in the aorta of the animals of this group was almost three times higher than in the rats which received NaCl alone. Because of the considerable scatter of the data, the cyclic AMP concentration did not differ significantly from that in the control series. The cyclic AMP/cyclic GMP ratio was only one-third as high as in the rats receiving salt, and it was significantly lower than in the intact animals.

Following administration of indomethacin in small doses the cyclic AMP/cyclic GMP ratio in the aortic wall was thus reduced. It must be pointed out that a similar tendency was observed by the writers both in the blood plasma and in the kideny tissue of these same rats [3]. To elucidate the cause of this phenomenon, adenylate cyclase (AC) and cyclic AMP-PDE activity was investigated in homogenates of the aorta of three series of rats: intact, receiving salt, and receiving a combination of indomethacin and salt. The investigations showed that AC activity did not differ significantly in all tissue samples tested (Fig. 2A). PDE activity showed a tendency to decline in the rats receiving salt and was significantly higher in animals receiving indomethacin (Fig. 2B). These findings explain the increase in the tissue cyclic AMP concentration in rats receiving salt and the opposite tendency observed in animals receiving indomethacin.

The causes of activation of cyclic AMP hydrolysis by indomethacin have not yet been explained. This phenomenon may perhaps be connected with accumulation of cyclic GMP in the cell. It has been shown that activity of one of the cyclic AMP phosphodiesterases is dependent on a Ca^{++}-regulated protein activator [8]. Stimulation of enzymic hydrolysis of cyclic AMP in the presence of an excess of cyclic GMP may thus be mediated through Ca^{++}, the intracellular level of which rises with an increase in the guanylate cyclase content [9]. It can be tentatively suggested that the disturbance of CN metabolism in these experiments also is connected with a corresponding change in PG metabolism: In experiments on the same series of rats large doses of indomethacin led to a sharp fall in the PG level of all classes, whereas in rats receiving 2 mg/kg indomethacin the $PGF_{2\alpha}$ concentration was increased and the PGE_2 level in the blood and aorta was lowered [2]. The experiments showed that the effect of PG on CN metabolism is mediated through a change in the activity of PDE, an enzyme to whose regulatory properties increasing importance is currently being attached.

The fall in the cyclic AMP/cyclic GMP ratio in the rat aorta was accompanied by a marked tendency toward vasoconstrictor responses and elevation of the blood pressure. Conversely, an increase in the contribution of cyclic AMP to CN metabolism, observed in the rats receiving salt, led to preservation of the tendency toward vasodilatation characteristic of intact animals. Similar changes in CN metabolism and vascular contractility have also been demonstrated by other workers in experiments on spontaneously hypertensive rats [6, 11].

These investigations demonstrate the role of the CN—RG system in the regulation of vascular tone. Biochemical, morphological, and functional changes discovered in the vessel walls of animals receiving small doses of indomethacin could be the cause of development of arterial hypertension.

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EFFECT OF APOMORPHINE ON OPIATE RECEPTORS IN THE RAT BRAIN

G. V. Morozov, L. F. Panchenko,
I. P. Anokhina, A. M. Balashov,
N. L. Vekshina, and O. S. Brusov

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Interest in the long familiar psychoparmacologic agent apomorphine has recently increased considerably. This is because of the recently discovered property of apomorphine of reducing withdrawal symptoms when the consumption of narcotics and alcohol is stopped and of reducing the dependence of chronic alcoholics and drug addicts on them [4]. A connection has also been found between the pharmacological action of apomorphine on animals in the laboratory, expressed as potentiation of some effects of morphine by apomorphine and inhibition of others [3, 6, 10].

Apomorphine has been shown to be an agonist of dopamine and to act on presynaptic dopamine receptors [3]. Morphine also is known to exert a marked influence on dopamine receptors. The connection observed between the effect of morphine and that of apomorphine is therefore nowadays explained by their interaction with these receptors [9]. Moreover, there is a tendency to explain most of the effects of apomorphine by its influence on the dopamine receptors of the brain [2]. However, there is some evidence which is not in harmony with the existing views [5, 14]. Analysis of these data, together with the well-known fact of the opiate activity of morphine, thus leads to the suggestion that apomorphine has a direct influence on the opiate system of the brain. Although in some investigations the question of the possible action of apomorphine on brain opiate receptors has been examined [13], no direct proof of this has so far been obtained.

The object of this investigation was accordingly to study interaction between apomorphine and the opiate receptors of the brain by direct methods.

EXPERIMENTAL METHOD

Male Wistar rats weighing 150-200 g were used.

To analyze the ability of apomorphine to bind with opiate receptors, methods of competitive replacement of the "pure" morphine antagonist naloxone [7,8(n)-^{3}H] or of the highly active D-analog of leucine-enkephalin — D-ala^2-[tyrosyl-3,5-^{3}H]-enkephalin (5-D-leucine) — from opiate receptors of the rat brain by apomorphine were used. The membrane fraction of the rat brain cells was obtained [11] as follows; a rat was decapitated, the brain was quickly removed in the cold, the cerebellum, which does not contain opiate receptors [7], was cut off, and the rest of the brain was homogenized in 45 volumes of cold Tris-HCl buffer, pH 7.7, in a homogenizer of Dounce type (glass—Teflon). The resulting suspension was centrifuged at 30,000g for 20 min at 4°C. The residue was suspended in the original volume of the same buffer and kept for 40 min at 37°C. The suspension was then recentrifuged under the same conditions. The residue thus obtained was resuspended in 50 mM Tris-HCl, pH 7.7 (25°C), containing 10^{-4} M EDTA, or in 50 mM Tris-HCl, pH 7.7 (25°C), containing

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