Acknowledgements—This work was supported by a grant from the Polish Academy of Sciences within the project II. 1.1.6.

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Biochemical Pharmacology, Vol 27, pp 2961–2962
Pergamon Press Ltd 1978 Printed in Great Britain

Effect of prostaglandin A_1, E_2 and $F_{2\alpha}$ on the monoamine oxidase (MAO) activity in rat liver and brain

(*Received* 8 *March* 1978; *accepted* 18 *April* 1978)

Prostaglandins are long-chain unsaturated fatty acids that have been found to be among the biochemical agents that provoke migraine attacks. They have been suggested to be involved in the mechanism of migraine [1]. Intravenous injection of PGE_1 and PGE_2 produces headache of the migraine type [2, 3]. Sandler [4] has postulated that small amounts of prostaglandins released into circulation could account for the vascular phenomenon of migraine by acting on receptors in the cerebral vascular bed. Prostaglandins are potent vasoactive substances probably contributing to the mechanism of pain during migraine in a variety of ways such as by sensitizing the response to other pain-producing stimuli [5] or by direct vasodilation [6]. These observations suggest the modes of action of prostaglandins in provoking the headache. It was thought worthwhile to study the effects of prostaglandins at the neurotransmitter monoamine level. Hence experiments were conducted to study the *in vivo* effect of prostaglandins on the enzyme monoamine oxidase (MAO) (EC 1.4.3.4; monoamine: O_2 oxidoreductase).

Norwegian rats weighing between 250–300 g were used in the present study. Prostaglandins (gift from Upjohn Company, Kalamazoo, Michigan) were first dissolved in ethanol (5 mg/ml) and made up to the required concentration (150 μg in 0.3 ml per injection) with sterile saline. Prostaglandins were injected s.c. at 1100 and 2300 hr everyday for 10 days. PGA_1, PGE_2 and $PGF_{2\alpha}$ were injected into groups of seven animals each. The control group received an equivalent amount of saline under similar treatment. The rats were sacrificed by stunning and decapitation. The liver and brain were removed immediately and homogenized in freshly prepared 0.5 M phosphate buffer pH 7.4. The cell debris including the nucleus was eliminated by centrifuging the crude homogenate in an International Refrigerated Centrifuge at 0° to 4° at 2500 rpm for 15 min. The supernatant was made up to the appropriate volume (20 m/g wt of liver and 40 ml/g wt of brain) with the buffer. This was used as the enzyme preparation for determining the activity of MAO with respect to its different substrates. The enzyme (1 ml) was incubated with 0.4 ml of 0.5 M phosphate buffer pH 7.4 and 1 ml of substrate (600 μg/ml of serotonin or 260 μg/ml of tyramine) for 1 hr, at the end of which period 0.6 ml of 25% TCA was added and chilled immediately to terminate the incubation. Control tubes were pretreated with TCA prior to the addition of the enzyme preparation for determining the activity at zero hour. The tubes were centrifuged and serotonin was estimated in the supernatant by the method of Udenfriend, Weissbach and Clark [7] and tyramine by the method of Udenfriend and Cooper [8]. The pellet was dissolved in 0.1 N NaOH and protein estimated by the method of Sutherland *et al* [9].

The results are shown in Tables 1 and 2. The MAO activity in liver with both tyramine and serotonin as substrates showed an increase with all the three prostaglandins tested, viz., PGA_1, PGE_2 and $PGF_{2\alpha}$. With tyramine as substrate, the increase in activity was highest with PGE_2, it being nearly 70 per cent. When serotonin was used as the substrate, there was an almost equal increase in activity with PGA_1, PGE_2 and $PGF_{2\alpha}$, the increase being approximately 57 per cent. The MAO activity in brain also showed an increase with both the substrates on treatment with PGA_1, PGE_2 and $PGF_{2\alpha}$. The increase with tyramine as substrate was $PGF_{2\alpha} > PGE_2 > PGA_1$. $PGF_{2\alpha}$ treated rats showed a 60 per cent increase in brain MAO activity. With serotonin as substrate, the increase was approximately 54 per cent with

Table 1. Effect of the various prostaglandins on the MAO activity in rat liver utilizing tyramine and serotonin as substrates

	Tyramine (Sp. Act. ± S.E.)	Serotonin (Sp. Act. ± S.E.)
Control (7)	10.68 ± 0.26	14.19 ± 0.24
PGA_1 (7)	13.63 ± 0.16	22.65 ± 0.31
PGE_2 (7)	17.37 ± 0.30	22.11 ± 0.31
$PGF_{2\alpha}$ (7)	15.59 ± 0.32	23.14 ± 0.22

The numbers in brackets indicate the number of experiments conducted in each case. $P < 0.001$ in all the cases.

Table 2. Effect of the various prostaglandins on the MAO activity in rat brain utilizing tyramine and serotonin as substrates

	Tyramine (Sp. Act. ± S.E.)	Serotonin (Sp. Act. ± S.E.)
Control (7)	23.27 ± 0.19	37.40 ± 0.61
PGA_1 (7)	26.82 ± 0.41	56.87 ± 0.64
PGE_2 (7)	33.42 ± 0.27	60.05 ± 0.63
$PGF_{2\alpha}$ (7)	37.40 ± 0.07	52.19 ± 0.41

The numbers in brackets indicate the number of experiments conducted in each case. $P < 0.001$ in all the cases.

PGA_1, 60 per cent with PGE_2 and 40 per cent with $PGF_{2\alpha}$.

The monoamine oxidase activity was expressed in terms of specific activity, i.e. μg substrate utilized/mg protein. It is clear from our results that there is an increase in the activity of MAO in liver and brain on subcutaneous injections of prostaglandins A_1, E_2 and $F_{2\alpha}$. This increase in enzyme activity was observed with respect to serotonin as well as tyramine as substrates, thereby implicating a depletion of these amines in their storage depots, viz., tissues like liver and brain. This would lead to a decrease in the circulating level of these amines. The vasodilatory property of prostaglandins has been well established [10–13]. This vascular effect may be due to a decreased level of amines. Our results suggest that prostaglandins-induced headache, therefore, may be due to a depletion of amines which is brought about by an increase in the activity of monoamine oxidase.

In summary, prostaglandins are vasoactive substances, known to produce vasodilation. Intravenously administered PGE_1 and PGE_2 produce migraine headache. Prostaglandin was injected subcutaneously (150 μg in 0.3 ml per injection) at 1100 and 2300 hr everyday for 10 days. The MAO activity was measured in liver and brain using serotonin as well as tyramine as substrates. The increase in activity was significant with both the substrates in both the tissues, viz., liver and brain. The prostaglandins tested were PGA_1, PGE_2 and $PGF_{2\alpha}$ and the increased activity was observed in all the cases. Our results suggest that the depletion of amines produced by an increase in the activity of MAO may account for the headache induced by prostaglandins.

Acknowledgements—We wish to thank Dr. C. H. Chakrabarti for providing the necessary facilities, and Mr. P. K. Mehta for technical assistance in carrying out the research work. The work was financially supported by CSIR (Council of Scientific and Industrial Research, India).

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Biochemical Pharmacology, Vol 27 pp 2962–2965 0006-2952/78/1215-2962 $02.00/0

Pharmacological augmentation of acetylcholine levels in kainate-lesioned rat striatum

(*Received* 23 *December* 1977; *accepted* 31 *March* 1978)

Injection of kainic acid (KA), a conformationally restricted analogue of glutamic acid [1], into the rat striatum produces a complete degeneration of neurons, the cell bodies of which are within a 1.5 mm radius of the injection site, but spares axons from extrinsic neurons [2]. The striatal kainate lesion results in a marked reduction in pre-synaptic markers for cholinergic neurons in the striatum, including the activity of chlorine acetyltransferase, the levels of endogenous acetylcholine (ACh), and the activity of the synaptosomal high affinity uptake process for choline; the pre-synaptic