Biochemical Pharmacology, Vol. 31, No. 9, pp. 1783–1784, 1982.
Printed in Great Britain.

0006-2952/82/091783-02 $03.00/0
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SHORT COMMUNICATIONS

Lowering of brain levels of the depressant prostaglandin D_2 by the anti-depressant tranylcypromine

(*Received* 3 *July* 1981; *accepted* 20 *October* 1981)

Tranylcypromine (TCP) is a monoamine oxidase (MAO) inhibitor and is used clinically as an anti-depressant. In 1976, Gryglewski *et al.* [1] reported that high *in vitro* doses of TCP inhibited arterial formation of prostaglandin I_2, a prostaglandin (PG) with vasodilator and platelet anti-aggregation properties. More recently, studies have shown that TCP also inhibits PG synthesis in platelets and inhibits arachidonic acid (AA) release from aortic endothelial cells [2, 3]. Prostaglandin D_2 has been reported to be the major PG in rat and gerbil brain [4, 5], and PGD_2 has been shown to cause sedation in monkey [6, 7] and cat [7], to prolong pentobarbital sleeping time in mice [8], and to modulate cyclic-AMP in mouse neuroblastoma cells [9]. These findings suggest the hypothesis that the anti-depressant effect of TCP may be mediated partly via an inhibition of depressant brain PG, perhaps PGD_2. The purpose of this study was to examine PGD_2 synthesis in mouse brain and to determine if *in vivo* doses of TCP can alter brain synthesis of PGD_2.

Since studies of the depressant action of PGD_2 have been conducted in mice [8], we chose the mouse as the experimental animal. To determine if PGD_2 was the major PG synthesized from the PG precursor AA in mouse brain, as has been reported for other rodents, we incubated tritiated AA with mouse brain homogenates and then analyzed for radioactive PGs. Male mice were decapitated and the whole brain minus the cerebellum was removed and homogenized in pH 7.4 Krebs–Ringer bicarbonate with dextrose at a tissue-to-volume ratio of 2.5 g/10 ml. Each incubation flask contained 10 ml of homogenate, 3 μCi of [^{3}H]AA (New England Nuclear, Boston, MA) and 0.5 μg of unlabeled AA (Nu Chek Prep, Elysian, MN). The homogenates were incubated for 2 hr in a metabolic shaker gassed with 95% O_2–5% CO_2. These incubation conditions were chosen to maximize incorporation of exogenous AA, which in similar experiments in brain tissue from other species has been reported to be very low [11]. Following incubation, the PGs were extracted and separated using high pressure liquid chromatography (HPLC), as we have previously reported [12].

In the second part of this study, mice were injected (i.p.) with saline vehicle or 5 or 50 mg/kg TCP 1 hr before they were killed, and the brain tissue was removed, incubated for 2 hr, and analyzed for PGD_2 synthesis from endogenous AA. Some brain incubates, which were prepared from animals that had received 50 mg/kg TCP, also had 500 μg/ml TCP added to the homogenates. The doses of TCP were chosen because 500 μg/ml has been reported to be the ID_{100} for PGI_2 synthesis [1] and 50 mg/kg has been shown to facilitate *in vivo* platelet aggregation in the mouse brain microcirculation [10], possibly via inhibition of PGI_2 synthesis in cerebral microvessels. Incubation conditions were the same as in the [^{3}H]AA experiments. To analyze PGD_2 formed from endogenous AA, we utilized HPLC and gas chromatography mass spectrometry, as previously reported [12].

The conversion of exogenous [^{3}H]AA into all PGs was 1.2%. Table 1 shows that PGD_2 was the major PG formed from [^{3}H]AA by the mouse brain homogenates. Because PGE_2, 6-keto-$PGF_{1\alpha}$ and thromboxane B_2 co-chromatograph in the HPLC system we used, and the counts in this combined peak were generally low, it was not possible to re-chromatograph these PGs and determine the exact ratio of these three cyclooxygenase products. Table 2 shows that the synthesis of PGD_2 from endogenous AA in control mouse brain was great and was statistically significantly decreased by pretreating animals with TCP.

Our results further strengthen the suggestion that rodent brain, in general, makes predominantly PGD_2. While TCP markedly decreased PGD_2 levels in mouse brain homogenates, whether TCP might decrease PGD_2 levels in intact tissue is uncertain. Unfortunately, PG levels in intact, quick frozen tissue are much lower than in incubates and often are below the level of sensitivity achieved in many PG assays.

The major anti-depressant action of TCP is undoubtedly through MAO inhibition; however, the current studies allow the hypothesis that part of the action of TCP may be via a reduction in PGD_2. This is especially true since PGD_2 has been reported to cause sedation in monkeys and cats [6, 7], prolong pentobarbitol sleeping time in mice [8], and reduce sympathetic neurotransmission [13].

Our data show that PGD_2 is the major cyclooxygenase product in mouse brain and that mouse brain PGD_2 synthesis is inhibited by the monoamine oxidase inhibitor tranylcypromine. More generally, this study enhances the hypothesis that PGs are modulators of neural activity and suggests that some CNS drugs may be acting partly via their effect on the levels of these modulators.

Table 1. Metabolism of tritiated AA in mouse brain homogenates*

Product	Percent
PGD_2	58.4 ± 11.2
$PGF_{2\alpha}$	20.3 ± 7.0
PGE_2 + 6-keto-$PGF_{1\alpha}$ + TxB_2	21.3 ± 3.0

* Values shown are the percent distribution of the radiolabeled products. Each value is the mean ± S.E.M. of ten experiments. Thromboxane B_2 (TxB_2) is the stable metabolite of thromboxane A_2, and 6-keto-$PGF_{1\alpha}$ is the stable metabolite of PGI_2.

Table 2. Effect of tranylcypromine on PGD_2 levels in mouse brain homogenates*

Dose TCP (mg/kg)	PGD_2, $\bar{X}$ ± S.D. (ng/g wet wt)
0	2134 ± 977
5	1292 ± 491
50	693 ± 315†
50 + 500 μg/ml	610 ± 334†

* Each group had an N = 5. Analysis of variance showed a significant effect of TCP at the P = 0.007 level.

† $P < 0.01$ by the Bonferroni test.

Acknowledgements—We thank the Upjohn Co. for authentic prostaglandins, Ms. R. Harris for secretarial assistance, and Mr. J. Saady and G. Nelson for technical assistance. Supported by NIH HL 18932 and NS 12587.

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Biochemical Pharmacology, Vol. 31, No. 9, pp. 1784–1787, 1982.
Printed in Great Britain.

0006-2952/82/091784-04 $03.00/0
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Promotion of membrane resealing by local anesthetics

(*Received* 27 *June* 1981; *accepted* 14 *October* 1981)

The spontaneous repair of membrane defects induced by mechanical trauma or osmotic shock is an important but little-understood ability of red cells. Most observations have been incidental to studies of red cell ghost membranes in which lysed and resealed ghosts were used to study various structural and transport features of the membrane. We have reported the detailed temperature dependence of resealing [1, 2] and found that the curve is sigmoidal, suggesting that resealing is a highly cooperative process. This temperature dependence could be dependent on a phase transition in a particular lipid region of the membrane [3]. To investigate this possibility, we have studied the effects of various membrane fluidizing compounds on resealing. We found that cationic amphiphiles increased resealing, while other fluidity-altering agents did not affect resealing.

Materials and methods

Ghost preparation and measurement of resealing using hemoglobin as a marker have been described [1, 2]. Briefly, mixtures of unsealed ghosts and hemoglobin are held at 0° for 10 min to permit hemoglobin to equilibrate with the ghost interior volume. The mixture is then brought to the desired final temperature and salt concentration simultaneously. After appropriate incubation times, resealing is stopped by adding a large volume of cold isotonic buffer, and trapped hemoglobin is determined. To assay drug effects, the compounds were added to the ghost–hemoglobin mixture during the preequilibration period at 0°. The volatile anesthetics were tested in sealed tubes to minimize evaporative loss, but we did not directly assay these compounds [4]. All compounds were obtained from the Sigma Chemical Co., St. Louis, MO, with the following exceptions: ether and chloroform (Mallinckrodt, St. Louis, MO) and halothane (Ayerst, New York, NY). For cholesterol depletion of red cells, the methods of Gottlieb [5] were used. Ghosts were prepared and resealing experiments were as described. The amounts of cholesterol [6] and phospholipid [7] were determined by standard methods.

Results

Anesthetics. The local anesthetics, dibucaine and tetracaine, were able to induce red cell ghost resealing at low temperatures. A typical result for dibucaine is shown in Fig. 1. Since amphipathic compounds are believed to fluidize lipid bilayers or to induce phase changes, it seemed possible that these results were related to changes in lipid bilayer properties. To test this, the extent of resealing after 1 hr at 10° was measured for ten amphipathic compounds. This temperature was chosen since resealing is ordinarily very slow at 10°, but, as Fig. 1 shows, dibucaine, and anesthetic was able to cause resealing at 10°. The compounds were chosen because studies are available of their fluidizing effects on bilayers and, in some cases, on erythrocyte membranes. Figure 2 shows typical results; the data are summarized in Table 1.

The effects of cationic amphiphiles on resealing were biphasic, i.e. as concentrations were increased, the percentage of ghosts resealed went through a maximum and declined (Fig. 2). This is similar to the effects of these compounds on erythrocyte osmotic fragility [8], where low concentrations of amphiphiles protect red cells against osmotic hemolysis, but higher levels promote hemolysis. Comparison between the concentrations of the drugs needed for 50% anti-hemolysis [8] and the concentrations