Biochemical Pharmacology, Vol. 33, No. 20, pp. 3315–3318, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

COMPARISON OF THE PROSTANOID SYNTHESIZING CAPACITY IN HOMOGENATES FROM PRIMARY NEURONAL AND ASTROGLIAL CELL CULTURES

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(Received: 16 April 1984; accepted: 23 July 1984)

The presence (1) and synthesis of prostanoids under in vitro (2-10) and in vivo (11-15) conditions has been well established in the CNS of several species including man (16). These results, however, do not permit to draw conclusions on the neuronal or extraneuronal origin of prostanoids, except for prostaglandin (PG) I_2, shown to be formed principally in blood vessels (6,16,17). Informations on their site of origin may be helpful to better understand the role of PGs and thromboxane (TX) in the CNS. As a model to examine the involvement of neuronal and/or glial elements in brain PG and TX biosynthesis we have chosen primary brain cultures. In this communication, we report on our first results concerning prostanoid formation in homogenates of primary neuronal and astroglial cell cultures.

METHODS and MATERIALS

Cell cultures: Cultures of astrocytes were prepared according to Booher and Sensenbrenner (18) with some modifications. Cerebral hemispheres from newborn Whistar rats were freed from their meninges, washed and cut into small pieces in the culture medium, which contained Dulbecco's modified Eagle Medium (DMEM), fetal calf serum (10%), $NaHCO_3$ (2g/l), penicillin (100U/ml) and streptomycin (100ug/ml). The tissue was dissociated by aspirating it ten times with a serological pipette. Afterwards, the suspension was centrifuged at 500 x g for 5 min and the resuspended pellet was passed through a nylon mesh (50µm pore size). This step was repeated again and the suspension was then washed and centrifuged twice at 100 x g for 5 min. Five ml of the cell preparation, adjusted to a final concentration of $5x10^5$ cells/ml, were seeded in 60 mm Falcon plastic dishes, and incubated at 37°C in a 95%/5% mixture of atmospheric air and CO_2. The medium was changed after 3 days and subsequently twice a week. The cultures reached confluency between 7 and 10 days. After 14 days they consist predominantly of mature and immature astrocytes with up to 3% oligodendroglia. Neuronal and fibroblast contamination was below 1%. Fourteen day old cultures were used in the experiments.

Cultures of neurones were prepared as described by Yavin and Yavin (19) with some modifications, using the whole cephalic region of rat embryos at 16 days gestation. The culture medium was: DMEM supplemented with glucose (6g/l), glutamate (0.3g/l) and insulin (80U/ml), inactivated horse serum (5%), with bicarbonate and antibiotics as above. After triturating and passing through a nylon mesh (50µm pore size), the suspension was centrifuged at 280 x g for 5 min, and the resuspended pellet was sieved again. All of the subsequent steps were the same as described above for astrocytes, except that the dishes were precoated with poly-l-lysin (10µg/ml) (Type VII B, Sigma). After three days one ml serum free medium, containing $2x10^{-5}$ M cytosine arabinoside (20) was added(final cc.:$3.3x10^{-6}$ M). Cells were kept in this medium without change up to one week. The cells sent out fibers after a few hours, and formed a network within 3 days. After seven days, a dense network of bipolar and pyramidal neurones developed. Contaminations with

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glioblasts (5-10%) and oligodendroglia (up to 2%) could be detected. Seven day old cultures were used in the experiments. Detailed morphological and immunocytochemical characterization of the neuronal and glial cell cultures will be published elsewhere (in preparation).

Determination of prostanoid-forming capacity: The cultures were washed twice with ice-cold Dulbecco's phosphate buffered saline (DPBS) including 0.1% glucose, and scraped off the dishes into small homogenization tubes in 2 x 1.5 ml of the the same buffer. After centrifugation at 300x g for 10 min, the cells were resuspended in 1 ml 0.1M tris-HCl buffer (pH 7.4) and homogenized in a glass teflon homogenizer with 800 rpm at 4°C for 1 min. Protein concentration of the homogenate was 0.24 ± 0.02 mg/ml (n=54) for neuronal cells, and 0.65 ± 0.02 mg/ml (n=190) for the glial cells, respectively, as determined by the method of Lowry et al. (21). The reaction mixture consisted of 0.9 ml homogenate, and ocassionally noradrenaline, pargyline or arachidonic acid in a total volume of 1 ml. Incubation was carried out at 37°C for 20 min in a shaking water bath, and stopped by addition of 1 ml ice-cold PBS containing 10^{-4} M indomethacin(IPBS). Corresponding blanks were prepared by adding IPBS before incubation. The samples were then filtered using Millipore membrane filters of 0.45 µm pore size. The filtrates were analysed for PGD_2, $PGF_{2\alpha}$, PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 by specific radioimmunoassays, as described previously (see 10). PG and TXB_2 formation was calculated by subtracting the individual blank values from the values found after incubation and expressed as ng/mg/20min. Differences were tested for statistical significance by Student's-t-test.

Materials: Tritiated prostanoids were purchased from New England Nuclear, Dreieich FRG. Pargyline HCl and l-Noradrenaline HCl were from Sigma St. Louis. Indomethacin (Merck, Sharp and Dohme, Rahway, N.I.) was dissolved in 0.1 M sodium phosphate buffer, pH 7.4 .

RESULTS and DISCUSSION

A time-dependent formation of cyclooxygenase products occured in homogenates of astrocyte cultures (Fig 1). The predominant metabolite was PGD_2 (10-17 ng/mg protein/20 min). Less TXB_2, $PGF_{2\alpha}$ and PGE_2 were synthetized (Fig 1., Table 1). No 6-keto-$PGF_{1\alpha}$ was detected. The synthesis of all cyclooxygenase metabolites could be enhanced by $5x10^{-5}$ M noradrenaline (NA) (Table 1 and 2). The stimulatory effect of NA was prevented by the monoamine oxidase inhibitor pargyline (10^{-4} M) (Table 2). The formation of all prostanoids was similarly affected by NA (Table 1) and pargyline, most probably by influencing the activity of cyclooxygenase, rather than that of the individual endoperoxide-converting enzymes.

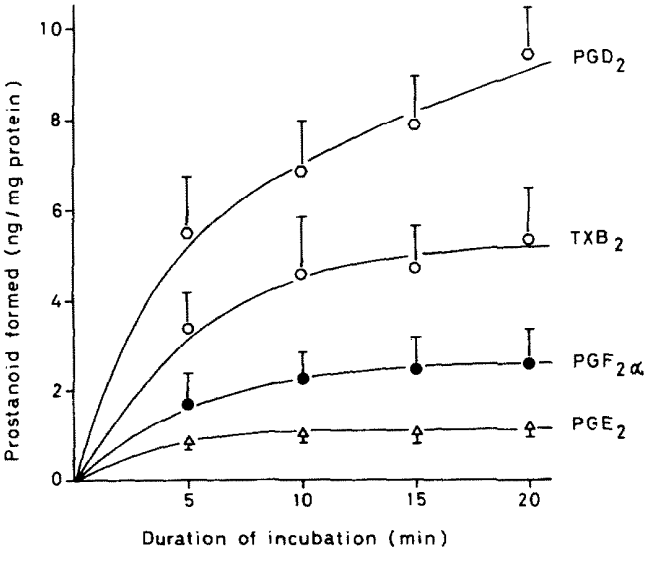

Fig.1. Time course of prostaglandin and thromboxane formation in homogenates of glial cell cultures. Results are the means ± S.E.M., n=14 from seven independent cultivations (incubations performed in duplicate).

Table 1. Prostanoid formation in homogenates of primary astroglial cell cultures in the presence and absence of noradrenaline.

	Prostanoid formed (ng/mg protein/20 min)			
	PGD_2	$PGF_{2\alpha}$	PGE_2	TXB_2
Control	17.03 ± 1.77	3.58 ± 0.41	3.35 ± 0.23	4.64 ± 0.92
Noradrenaline ($5x10^{-4}$ M)	26.27 ± 1.49***	7.90 ± 1.07***	6.69 ± 0.82***	16.11 ± 2.90**

Results are the means ± S.E.M., n=16 from four independent cultivations (incubations performed in quadruplicate).
*** $p < 0.001$; ** $p < 0.005$ v.s. control by Student's t-test
The amounts of prostanoid-like substances present in the blank incubations (see methods) were (ng/mg protein): PGD_2-like: 1.58 ± 0.13, $PGF_{2\alpha}$-like: 0.70 ± 0.08, PGE_2-like: 0.40 ± 0.06 and TXB_2-like: 0.68 ± 0.11 (n=16).

Table 2. Inhibition of the noradrenaline-stimulated cyclooxygenase activity by pargyline in homogenates of rat primary astrocyta cultures

	Cyclooxygenase activity+ (ng prostanoid formed/mg protein/20 min)	
	None	Noradrenaline($5x10^{-4}$ M)
Control	32.23 ± 2.47	59.43 ± 4.07
Pargyline (10^{-4} M)	21.57 ± 2.23**	28.23 ± 2.17***

Results are the means ± S.E.M., n=12 from four independent cultivations (incubations performed in triplicate).
+Calculated as the sum of PGD_2, $PGF_{2\alpha}$, PGE_2 and TXB_2 formed
*** $p < 0.001$; ** $p < 0.005$ v.s. corresponding control by Student's t-test

Summarizing these results, astrocyte homogenates produced from endogenous substrate a similar pattern of prostanoids in the same order of magnitude as whole brain homogenates did (5,6,10). Their cyclooxygenase activity could be stimulated with noradrenaline in a monoamine oxidase dependent way, as found earlier for total brain homogenates (7-10). Thus this cell type displays all of the known characteristics of brain prostanoid formation in vitro. This suggest, that even in whole brain homogenates, astrocytes may be responsible for a considerable part of cyclooxygenase activity. Although, obtained in homogenates of primary cell cultures, these results, along with the findings on the presence of cyclooxygenase in a glioma cell line (22) and in Bergman glia cells (23), direct the attention to a possible role of glial elements in prostanoid-mediated physiological or pathological processes in the CNS. The fact, that astrocytes are a powerful source of PGD_2, might lead to some new ideas in explaining effects of this putative neuromodulator substance (24,25).

Table 3. Cyclooxygenase activity in homogenates of primary neuronal cell cultures under different conditions.

	Cyclooxygenase activity+ (ng prostanoid formed/mg protein/20 min)		
	None	Arachidonic acid(10^{-6} M)	Arachidonic acid(10^{-5} M)
Control	0.52 ± 0.25	2.57 ± 0.92	2.47 ± 0.71*
Noradrenaline ($5x10^{-4}$ M)	1.45 ± 0.61	2.72 ± 0.82*	2.91 ± 0.99*

Results are the means ± S.E.M., n=6 from three independent cultivations (incubations performed in duplicate
+Calculated as the sum of PGD_2, $PGF_{2\alpha}$, PGE_2 and TXB_2 formed
* $p < 0.05$ v.s. control (without arachidonic acid and noradrenaline) by Student's t-test
The amounts of prostanoid-like substances present in the blank incubations (see methods) were (ng/mg protein): PGD_2-like: 0.18 ± 0.06, $PGF_{2\alpha}$-like: 0.25 ± 0.05, PGE_2-like: 0.15 ± 0.02 and TXB_2-like: 0.16 ± 0.02 (n=8).

Homogenates of neuronal cell cultures did not exhibit significant prostaglandin or thromboxane synthesis during incubation. Only 0.17 ± 0.08 ng PGD_2, 0.27 ± 0.14 ng $PGF_{2\alpha}$, 0.05 ± 0.02 ng PGE_2 and 0.04 ± 0.02 ng TXB_2 were formed per mg protein and 20 min. Even after stimulation with arachidonic acid and NA the amounts of prostanoids obtained were still extremely small compared to prostanoid production of astrocytes (Table 3). In contrast to these results remarkable cycloxygenase activity (22,26) as well as PGD_2-synthetase activity (24) was found in homogenates of certain neuroblastoma cell lines. Therefore, caution is needed in interpreting our results obtained with the primary neuronal cell cultures, which seem to be a very week source of cyclooxygenase.

In conclusion, cultured astrocytes are capable of producing large amounts of prostanoids, especially PGD_2, the major cyclooxygenase product in the rodent brain. On the other hand, cultured neuronal cells possess surprisingly low cyclooxygenase activity. Results of our experiments in progress with intact astrocytes and neurones in culture show the same difference between prostanoid production of the two cell types (in preparation). The characterization of the prostanoid-forming system of astrocyte cultures and the unexpected phenomenon obtained with the primary neuronal cell cultures in more detail requires further research.

The excellent technical assistance of Miss.A. Nagel is gratefully acknowledged. We wish to thank Dr.U. Förstermann for the helpful discussions during this work.This study was supported by the Deutsche Forschungsgemeinschaft (SFB 70).

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