

Acute hypoxia modulates arachidonic acid metabolism in cat carotid bodies. Role of dopamine

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Abstract. The hypoxic stimulus of the arterial blood is transformed at the carotid body (CB) chemoreceptors into neuronal signals regulating respiration. The mechanism of chemotransduction is until now not well understood. In this study the regulation of arachidonic acid (AA) release and its incorporation into membrane glycerolipids were investigated. Moreover, the effect of hypoxia and dopamine (DA) on these processes was evaluated. The CB were excised from cats exposed in situ to normoxia or hypoxia. Then CB were homogenized and used as a source of enzyme (s). It was observed that Ca^{2+} enhanced the release of AA by 40-50% through the action of phospholipase C together with diacyl-glycerol lipase and phospholipase A_2 . Acute hypoxia significantly decreased AA incorporation into phosphatidylinositol (PtdIns) and enhanced the level of AA radioactivity in diacylglycerol and AA-CoA. These results suggest that hypoxia induces inhibition of AA incorporation on the level of acyl-CoA-lysophospholipid:acylotransferases. DA decreased AA incorporation into PtdIns and exerted an additive inhibitory effect in hypoxic samples. These results demonstrate that AA metabolism in CB is significantly affected by hypoxia and that DA is not responsible for the hypoxia-induced alteration of lipid metabolism in CB.

Key words: arachidonic acid, carotid bodies, dopamine, hypoxia

INTRODUCTION

Despite widespread interest in the neural control of respiration, the cellular and molecular mechanisms which mediate afferent transmission from peripheral chemoreceptors to brain stem respiratory neurones remain poorly understood.

The mechanism of chemoreception in the carotid body is often connected with the release of some neurotransmitters, particularly dopamine and with the action of Ca^{2+} and K^{+} channel function (Fidone and Gonzalez 1986, Hescheler et al. 1989, Lopez-Lopez et al. 1989, Urena et al. 1989, Biscoe and Duchon 1990, Gonzalez et al. 1992). Hypoxia, a natural CB stimulus, induces dopamine release (Fidone et al. 1982, Shaw et al. 1989).

The role of dopamine in chemoreception processes is controversial. Both inhibitory and excitatory effects on chemoafferent activity have been reported, depending on dose, species and experimental technique used (Okajima and Nishi 1981). Hypoxia and ischemia interfere with plasma membrane processes, in some tissues cause changes in membrane phospholipid composition and accelerate the breakdown of phospholipids as well as release of arachidonic acid (Bazan 1970, Bazan et al. 1971, Łazarewicz et al. 1972, Strosznajder and Sun 1981, 1982, Noremborg and Strosznajder 1986, Van der Vusse et al. 1994).

In our previous studies it was found that acute hypoxia influenced phosphoinositide metabolism in CB (Pokorski and Strosznajder 1992, 1993, Strosznajder 1995). These phospholipids in nervous tissue are enriched in arachidonic acid. A recent study indicated an active mechanism for phosphoinositides turnover and for recycling of AA in brain (Sun 1992). There is convincing evidence for the involvement of ischemia-stimulated hydrolysis of polyphosphoinositides in the initial phase of AA release in brain (Yoshida et al. 1986, Sun 1992).

Arachidonic acid is involved in stimulus-secretion coupling in a variety of cell types and there is a growing body of evidence that it modulates neurotransmission and signal transduction. Arachidonic acid has been shown to be a stimulator of the

release of transmitters from synaptosomal or brain slice preparations (Shimizu and Wolfe 1990, Dorman et al. 1992). Moreover, increased availability of unesterified AA has been correlated, depending on amount and location, with the induction of long term potentiation (LTP) (Lynch et al. 1989, Luo and Vallano 1995), Ca^{2+} mobilization and protein kinase C (PKC) activation (Shinomura et al. 1992). In addition to the above mentioned potent functions, arachidonic acid is a precursor for prostaglandins, thromboxanes and leukotrienes as well as for other eicosanoids (Shimizu and Wolfe 1990). These compounds may play important roles in the modulation of membrane function and signal transduction as well as in control of microcirculation (Siesjö and Katsura 1992). AA may be released by phospholipase A₂ (PLA₂) from the second position of glycerol moiety of glycerophospholipids, or by phospholipase C (PLC) followed by the action of diacylglycerol lipase (DAG-lipase) (Bell et al. 1979). Both PLA₂ and PLC may be directly coupled to transmitter receptors by GTP-binding proteins (Burch et al. 1986, Jelsema 1987, Axelrod et al. 1988, Farooqui et al. 1992), however, Ca^{2+} may be involved in activation of enzymes (Simon et al. 1986). Until now the arachidonic acid release, uptake and role in chemotransduction processes in CB are hardly known.

The aim of this study was to investigate the pattern of [$1\text{-}^{14}\text{C}$] arachidonic acid incorporation into glycerolipids of normoxic CB as well as the effect of acute hypoxia on arachidonic acid release and its incorporation into glycerolipid. Moreover, the role of dopamine in modulation of arachidonic acid metabolism was investigated.

METHODS

Chemicals

[$1\text{-}^{14}\text{C}$] arachidonyloglycerophosphatidylinositol ([$1\text{-}^{14}\text{C}$] AA-PtdIns or PtdIns) s.a. 57 mCi/mmol and [$1\text{-}^{14}\text{C}$] arachidonic acid s.a. 59 mCi/mmol were purchased from Amersham, U. K.

ATP, CoA, dithiothreitol (DTT), sodium deoxycholate (DOC), quinacrine, neomycine, GTP γ S,

were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Thin layer chromatography (TLC) and HPTLC plates were from Merck, Darmstadt Germany.

Animals and hypoxic treatment

Adult cats 2-3 kg body weight were used for these experiments. Cats were anaesthetized with chloralose - urethane in a dose of 30 and 800 mg/kg b.w. i.p. respectively, and exposed to exclusively normoxic ($\text{PaO}_2 \approx 90$ mmHg) or hypoxic ($\text{PaO}_2 \approx 20$ mmHg) gas mixture for 20 min at constant PaCO_2 and other acid-base variables. Then perfusion through the aorta with an ice-cold Krebs-buffer solution was performed. Carotid bodies were quickly excised and subsequently subjected to a short pre-incubation time with dopamine 100 μM in the presence of pargyline 10 μM , or homogenized in 20 mM Tris-HCl buffer pH 7.4 to be used as a source of enzyme(s) for the studies on arachidonic acid release and its incorporation into glycerolipid. Protein was determined according to the method of Lowry et al. (1951). Research project was accepted by the Commission of Ethic.

Assay of arachidonic acid release from [1- ^{14}C] arachidonyl-glycerophosphoinositol by enzyme(s) of normoxic and hypoxic CB

Carotid bodies homogenate was used as a source of enzymes (phospholipase A_2 and phospholipase C, DAG-lipase). [1- ^{14}C] AA-PtdIns was used as an exogenous substrate. [1- ^{14}C] AA PtdIns was introduced into the water phase after evaporation of organic solvent and addition of 1% DOC together with 100 mM Tris-HCl buffer, pH 7.4. Then it was vortexed for 2 min. Subsequently the substrate (PtdIns) in the amount of 20 nmol 30,000-40,000 dpm was added into each incubation tube. In final volume of 200 μl of incubation mixture the final concentration of DOC was 0.1%, Tris-HCl pH 7.8, 20 mM, and about 50 μg protein was added. In few experiments quinacrine (50 μM) or neomycine (1 mM) were added into the incubation mixture.

Assay of [1- ^{14}C] arachidonic acid incorporation into glycerolipids of normoxic and hypoxic cat CB

Arachidonic acid incorporation was assayed in an incubation system that contained 0.2 μCi of [1- ^{14}C] AA, 2.5 mM ATP, 0.1 mM CoA, 10 mM MgCl_2 , 0.3 mM DTT and 50 μg of CB homogenate protein in total volume of 200 μl . While determining the effect of dopamine, this agonist (100 μM) together with pargyline (10 μM) was added to the incubation mixture for AA incorporation. The AA incorporation was performed during 15 min incubation at 37°C in a shaking water bath. Then the reaction was terminated by addition chloroform:methanol mixture (1:2 by vol.) and the lipids were extracted.

Extraction of lipids and arachidonyl-CoA (AA-CoA)

Lipids were extracted according to the method of Bligh and Dyer (1959). The extraction procedure was performed at 0-4°C. The total lipid extract was evaporated to dryness from the samples under the stream of nitrogen and then was applied to silica gel TLC plates. The arachidonyl-CoA that remained in the Bligh and Dyer's upper aqueous phase was purified by three extractions of aqueous phase with 1 ml of n-heptane, each time centrifuged at 2,500 rpm for 10 min and then a 0.5 ml aliquot of the water phase was taken for the measurement of AA-CoA radioactivity using 10 ml of Bray's scintillation fluid. Radioactivity of samples was measured in a LKB Wallach-1409 scintillation counter.

Separation of lipids

Lipid extract from CB was separated in the presence of brain lipid extract on TLC by the separation-reaction-separation procedure of Horrocks and Sun (1972). A solvent system containing chloroform-methanol-acetone-acetic acid-0.1 M ammonium acetate 130:60:55:35:10 by vol. was used for development in the second dimension. Iodine vapour was used for detection of the lipid spots. In some experi-

ments the solvent system consisted of chloroform:methanol: 4N ammonium hydroxide 9:7:2 by vol. Free fatty acids (FFA) and DAG were separated on TLC using a solvent system consisting of chloroform: acetone 96:4 by vol. The lipid spots visualized in iodine vapour and corresponding to lipid standard were scraped into the scintillation vials. Radioactivity was counted using 10 ml of Brays scintillation fluid in LKB Wallach-1409 counter.

RESULTS

Effect of Ca^{2+} and hypoxia on enzyme(s) activity involved in arachidonic acid release from labelled phosphatidylinositol

Homogenate of normoxic and hypoxic CB was used as a source of enzymes in the studies on arachidonic acid release from exogenous substrate, labelled phosphatidylinositol [$1\text{-}^{14}\text{C}$] AA-PtdIns.

The substrate was degraded by CB homogenate enzymes into two radioactive products such as DAG and free radioactive AA. Seventy percent of radioactivity was found in DAG and about 30% in AA. Degradation of substrate and AA release were activated by increasing Ca^{2+} concentration. $\text{GTP}\gamma\text{S}$

had no effect on PtdIns degradation and AA liberation from this substrate (Fig. 1).

The results with PLC inhibitor neomycin (1 mM) and PLA2 inhibitor quinacrine (50 μM) indicate that both enzymatic processes were involved in [$1\text{-}^{14}\text{C}$]AA-PtdIns degradation and in liberation of DAG and AA (Fig. 2).

It was observed that hypoxia activates [$1\text{-}^{14}\text{C}$] AA-PtdIns degradation into DAG and AA by enzyme(s) of CB by about 115-140% vs. control value, however, results were not statistically significant (data not shown).

Preincubation of CB with DA had no significant effect on AA release (data not shown).

Effect of acute hypoxia and dopamine on incorporation of [$1\text{-}^{14}\text{C}$] arachidonic acid into glycerolipids of cat CB

Radioactive arachidonate was incorporated mainly into phosphatidylinositol (PtdIns), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidic acid (PA), diacylglycerol (DAG) and polyphosphoinositides (Poly-PtdIns) in normoxic CB. The pattern of AA incorporation into glycerolipid was

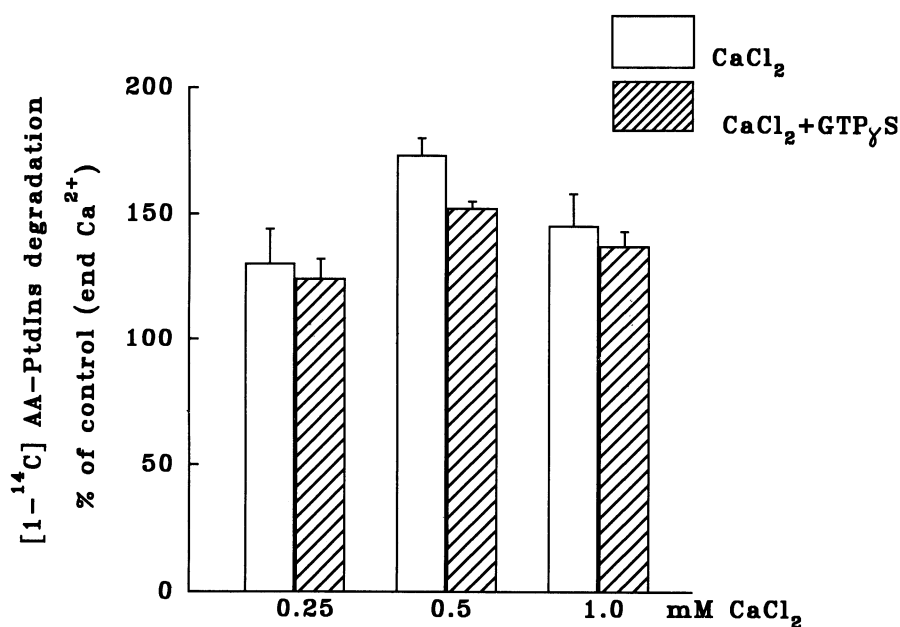


Fig. 1. Effect of Ca^{2+} and $\text{GTP}\gamma\text{S}$ on arachidonic acid release from [$1\text{-}^{14}\text{C}$]arachidonoylo-PtdIns. Ca^{2+} ions (0.25-1.0 mM) were added into incubation mixture containing about 50 μg protein of CB homogenate. $\text{GTP}\gamma\text{S}$ was added at 100 μM . Incubation was carried out for 30 min at 37°C in a shaking water bath. The data are means \pm SD from 3-4 experiments carried out in triplicate. The data are expressed as a percent of control (AA release in the presence of endogenous Ca^{2+}).

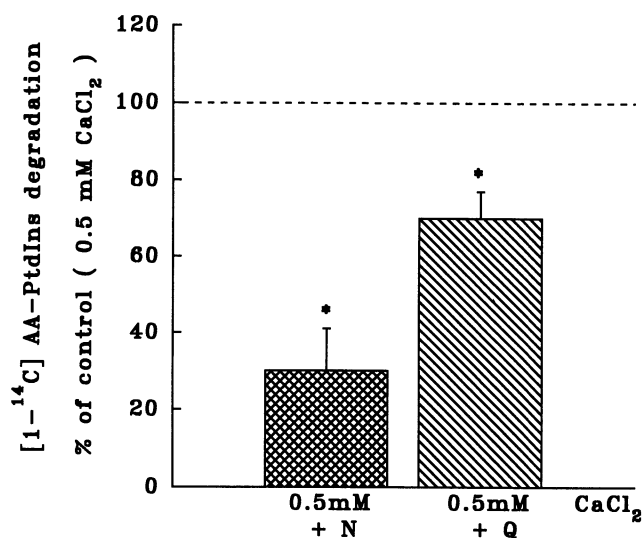


Fig. 2. Action of quinacrine (Q) and neomycine (N) on arachidonic acid release. AA liberation in the presence of 0.5 mM CaCl_2 was a control value. The data are means \pm SD from 3-4 experiments. The data are statistically significant from control value. * $P < 0.05$ by Student *t*-test.

significantly changed in hypoxic CB and it was as follows: $\text{PC} > \text{PS} > \text{PE} > \text{PtdIns} > \text{DAG} > \text{PA} > \text{PolyPtdIns}$ (Table I).

When radioactivity of the total phospholipids in normoxic CB was compared with respect to the total radioactivity of hypoxic or dopamine-treated normoxic samples, a significantly lower incorporation of the AA into the phospholipids was observed. The data in Table I present the percentage distribution of

AA radioactivity of particular glycerolipid in total analysed glycerolipid radioactivity.

The hypoxic samples indicated a decrease in the incorporation of $[1-^{14}\text{C}]$ AA radioactivity into PtdIns by about 50%. Dopamine added into control samples slightly decreased AA incorporation into PtdIns by about 18%. Dopamine added into the hypoxic samples additionally decreased AA incorporation into PtdIns. In hypoxia a significant increase in the level of $[1-^{14}\text{C}]$ AA radioactivity was observed in the fraction of diacylglycerides (1 fold) and also in phosphatidylcholine. Dopamine added to the hypoxic samples slightly increased the level of radioactivity into PC and PE as compared to the hypoxic condition (Table I).

The ratio of radioactivity between diacyl-GPI and DAG was significantly lower in the hypoxic samples as compared to control (Fig. 3).

Effect of acute hypoxia and dopamine on the level of arachidonyl-CoA radioactivity in CB

It was observed that in the hypoxic samples the level of AA-CoA radioactivity was significantly higher as compared to control (normoxic) samples of CB (Fig. 4).

The action of DA, opposite to hypoxia effect, decreased the level of AA-CoA radioactivity. When

TABLE I

Effect of dopamine and hypoxia on incorporation of $[1-^{14}\text{C}]$ arachidonic acid into glycerolipids of cat carotid bodies

| Lipids | % of total glycerolipids | | | |
|------------|--------------------------|------------------|-------------------|------------------|
| | Control | Control+DA | Hypoxia | Hypoxia+DA |
| PtdIns | 27.86 \pm 0.57 | 22.87 \pm 1.71 | *13.38 \pm 2.32 | 11.76 \pm 1.76 |
| PolyPtdIns | 4.57 \pm 0.46 | 4.54 \pm 0.25 | 4.24 \pm 0.44 | 3.82 \pm 0.72 |
| PA | 12.41 \pm 0.63 | 13.58 \pm 0.92 | 11.53 \pm 2.00 | 9.55 \pm 0.55 |
| PC | 20.29 \pm 0.76 | 18.08 \pm 2.12 | *28.32 \pm 2.86 | 32.33 \pm 0.81 |
| PS | 16.08 \pm 0.02 | 18.88 \pm 0.72 | 16.29 \pm 1.88 | 13.94 \pm 0.16 |
| PE | 13.80 \pm 0.94 | 16.66 \pm 1.40 | 14.51 \pm 1.24 | 17.50 \pm 0.59 |
| DAG | 5.32 \pm 1.67 | 5.63 \pm 0.96 | *11.63 \pm 0.89 | 11.08 \pm 0.38 |

The results are means \pm SD from 3-4 experiments carried out in triplicate. Hypoxia *versus* control * $P < 0.05$ by Student *t*-test.

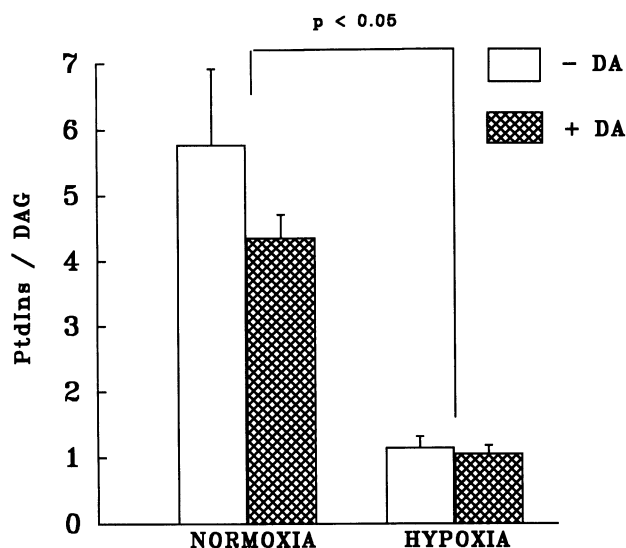


Fig. 3. The ratio of $[1-^{14}\text{C}]$ AA radioactivity of PtdIns versus $[1-^{14}\text{C}]$ AA radioactivity of diacylglycerols in normoxic and hypoxic cat carotid body. Effect of dopamine. Each value represents the mean from 3-4 experiments. $P < 0.05$ by Student *t*-test.

DA was added into the hypoxic samples there was a slightly lower level of AA-CoA radioactivity in the aqueous phase as compared to hypoxic samples, however, this was statistically insignificant (Fig. 4).

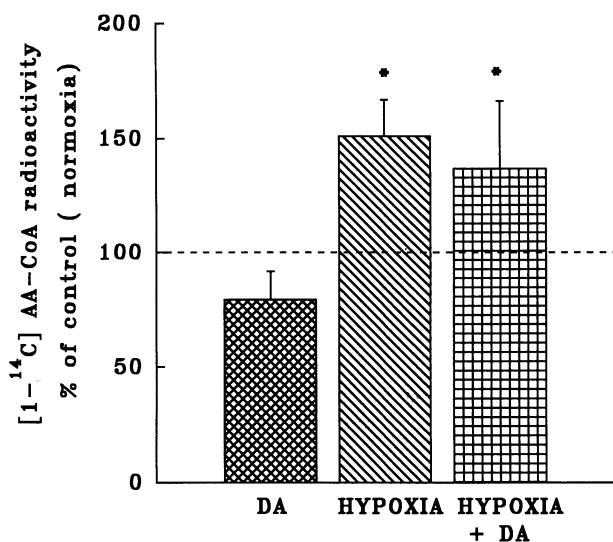


Fig. 4. Effect of hypoxia and dopamine on the level of $[1-^{14}\text{C}]$ AA-CoA radioactivity. Results expressed as a percentage of control (normoxia) represent the mean \pm SD from 3-4 experiments. The data are statistically significant from control value. * $P < 0.05$ by Student *t*-test.

DISCUSSION

These experiments indicate for the first time that acute hypoxia leads to a transient imbalance between lipid hydrolysis and (re)synthesis in cat carotid bodies.

The findings include:

- The enhanced degradation of exogenous $[1-^{14}\text{C}]$ AA-PtdIns into DAG and AA in the presence of Ca^{2+} by the action of phospholipase C together with diacylglycerol lipase and phospholipase A_2 .
- The significant and specific decrease of AA incorporation into phosphatidylinositol. The enhancement of the level of $[1-^{14}\text{C}]$ AA radioactivity in DAG. In consequence the significant decrease in PtdIns/DAG ratio indicates modulation of membrane glycerolipids of hypoxic CB.
- The increase of the level of radioactivity in arachidonyl-CoA.

Moreover, these data suggest the modulatory role of dopamine in arachidonic acid metabolism in normoxic and also in hypoxic carotid bodies.

These results indicate that hypoxia significantly affects AA and glycerolipid metabolism in CB and that dopamine plays a modulatory role in AA glycerolipid metabolism in normoxic and hypoxic CB. However, DA is not responsible for hypoxia-induced alteration of lipid metabolism in CB. These lipid changes seem to be involved in the mechanism of chemotransduction.

There are many controversies in the literature about phospholipases activity during hypoxia, ischemia reperfusion time in brain and in the heart. In *in vitro* studies higher and lower phospholipases activity was observed by Edgard et al. (1982) and Van der Vusse et al. (1994).

In the previous studies we have found that acute hypoxia specifically and significantly stimulated PtdIns(4,5) P_2 -PLC in CB but had negligible or no effect in superior cervical ganglia and brain stem, respectively (Pokorski and Strosznajder 1992, 1993, Strosznajder 1995).

It seems that in carotid bodies PLC acting on phosphoinositides together with DAG-lipase may

be more important for AA release than phospholipase A₂. Previously Gammon et al. (1989) have found that PLC and DAG lipase are mainly involved in AA release in superior cervical ganglia.

Net degradation of cellular phospholipids might be caused by altered (re)synthesis. Acute *in vivo* hypoxia significantly and specifically decreases the acylation of diacylglycerophosphoinositol and enhances the level of AA-CoA radioactivity. These results suggest that inhibition occurs at the level of the lysophospholipid:acyl-CoA acyltransferase. Kajiyama and co-workers (1987) have observed the inhibitory effect of ischemia on the activity of lysophosphatidyl-acyl-transferase (Van der Vusse et al. 1994). Acyl-CoA is usually maintained at a very low level in the cells because an excess of acyl-CoA in membrane may inhibit some enzyme(s) activity and physiological processes, and it may be detrimental to the cell membranes due to its potent detergent properties. Higher levels of this compound are very quickly removed by acyl-CoA hydrolyse; however, an elevated concentration of calcium ions may exert an inhibitory action on AA-CoA hydrolase (Strosznajder and Sun 1982). Nevertheless, metabolism of acyl-CoA, for example in brain synaptosomes, is complicated by the tendency of this compound to bind with Ca²⁺ and membrane phospholipids (Strosznajder and Sun 1982) and in this form acyl-CoA is not readily accessible to hydrolysis by acyl-CoA-hydrolase. The mechanism responsible for the accumulation of AA-CoA in hypoxic CB will be investigated further. These results may suggest that hypoxia exerts inhibitory action on acyltransferase activity and enhances the level of AA-CoA. However, it seems that dopamine decreases the turnover of AA and glycerolipid metabolism in CB through a mechanism different than that which occurs in hypoxia. Dopamine is not responsible for hypoxia evoked disturbances of AA-glycerolipid metabolism.

ACKNOWLEDGEMENTS

Author expresses thanks to Prof. J. Strosznajder and Prof. M. Pokorski for help and advice, Ms. Danuta

Kacprzak for excellent technical assistance, Mrs. Maria Izak for typing of this manuscript. This work was completely supported by grant of Polish Scientific Research Committee Project Nr 6 P207 029 05.

REFERENCES

- Axelrod J., Burch R.M., Jelsema C.I. (1988) Receptor-mediated activation of phospholipase A₂ via GTP-binding proteins: arachidonic acid and its metabolites as second messengers. *Trends Neurosci.* 11: 117-122.
- Bazan N.G. (1970) Effect of ischemia and electroconvulsive shock on free fatty acid pool in the brain. *Biochim. Biophys. Acta* 218: 1-10.
- Bazan N.G., Bazan H.E.P., Kennedy W.G., Joel C.D. (1971) Regional distribution and rate of production of free fatty acids in rat brain. *J. Neurochem.* 18: 1387-1393.
- Bell R.L., Kennerly D.A., Stanford N., Majerus P.W. (1979) Diglyceride lipase: a pathway for arachidonate release from human platelets. *Proc. Natl. Acad. Sci. USA* 76: 3238-3241.
- Biscoe T.J., Duchon M.R. (1990) Responses of type I cells dissociated from the rabbit carotid body to hypoxia. *J. Physiol.* 428: 39-59.
- Bligh E.C., Dyer W.J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37: 911-917.
- Burch R.M., Luini A., Axelrod J. (1986) Phospholipase A₂ and phospholipase C are activated by distinct GTP-binding proteins in response to adrenergic stimulation in FRTL5 thyroid cells. *Proc. Natl. Acad. Sci. USA* 83: 7201-7205.
- Dorman S.V., Hamm T.F.R., Damron D.S., Freeman E.J. (1992) Modulation of glutamate release from hippocampal mossy fiber nerve endings by arachidonic acid and eicosanoids. In: *Neurobiology of essential fatty acids. Advances in experimental medicine and biology* (Eds. N.G. Bazan, M.G. Murphy and G. Toffano). Vol. 318. Plenum Press, New York, p. 121-136.
- Edgar A.D., Strosznajder J., Horrocks L.A. (1982) Activation of ethanolamine phospholipase A₂ in brain during ischemia. *J. Neurochem.* 39: 1111-1116.
- Farooqui A.A., Hirashima Y., Horrocks L.A. (1992) Brain phospholipases and their role in signal transduction. In: *Neurobiology of essential fatty acid. Advances in experimental medicine and biology* (Eds. N.G. Bazan, M.G. Murphy and G. Toffano). Vol. 318. Plenum Press, New York, p. 11-25.
- Fidone S.J., Gonzalez C. (1986) Initiation and control of chemoreceptor activity in the carotid body. In: *Handbook of physiology. Section 3. The respiratory system* (Eds. N.S. Cherniack and J.C. Widdicombe). American Society Physiology, Oxford, p. 247-312.

- Fidone S.J., Gonzalez C., Yoshizaki K. (1982) Effects of low oxygen on the release of dopamine from the rabbit carotid body in vitro. *J. Physiol.* 333: 93-110.
- Gammon C.M., Allen A.C., Morell P. (1989) Bradykinin stimulates phosphoinositide hydrolysis and mobilisation of arachidonic acid in dorsal root ganglion neurons. *J. Neurochem.* 53: 95-101.
- Gonzalez C., Almaraz L., Obeso A., Riquel R. (1992) Oxygen and acid chemoreception in the carotid body chemoreceptors. *Trends Neurosci.* 15: 146-153.
- Hescheler J., Delpiano M.A., Acker R., Pietruschka F. (1989) Ionic currents on type-I cells of the rabbit carotid body measured by voltage clamp experiments and the effect of hypoxia. *Brain Res.* 486: 79-88.
- Horrocks L.A., Sun G.Y. (1972) Ethanolamine plasmalogens. In: *Research methods in neurochemistry* (Eds. R. Rodnight and N. Marks). Plenum Press, New York, p. 223-231.
- Jelsema C.L. (1987) Light activation of phospholipase A2 in rod outer segments of bovine retina and its modulation by GTP-binding proteins. *J. Biol. Chem.* 262: 163-168.
- Kajiyama K.L., Pauly D.H., Hughes H., Yoon S.B., Entman M.L., McMilin-Wood (1987) Protection by verapamil of mitochondrial glutathione equilibrium and phospholipid changes during reperfusion of ischemic canine myocardium. *Circ. Res.* 61: 301-310.
- Lopez-Lopez J.R., Gonzalez C., Urena J., Lopez-Barneo J. (1989) Low PO₂ selectively inhibits K channel in chemoreceptor cells of the mammalian carotid body. *J. Gen. Physiol.* 93: 1001-1015.
- Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.
- Luo Y., Vallano M.L. (1995) Arachidonic acid but not sodium nitroprusside stimulates presynaptic protein kinase C and phosphorylation of GAP-43 in rat hippocampal slices and synaptosomes. *J. Neurochem.* 64: 1808-1818.
- Lynch M.A., Errington M.L., Blise (1989) Nordihydroguaiaretic acid blocks the synaptic component of long term potentiation and the associated increases in release of glutamate and arachidonate. An in vivo study in the dentate gyrus of the rat. *Neuroscience* 30: 693-701.
- Łazarewicz J., Strosznajder J., Gromek A. (1972) Effects of ischemia and exogenous fatty acids on the energy metabolism in brain mitochondria. *Bull. Acad. Pol. Sci. Ser. Sci. Biol.* 20: 599-606.
- Negre-Aminou P., Pfenninger K.H. (1993) Arachidonic acid turnover and phospholipase A2 activity in neuronal growth cones. *J. Neurochem.* 60: 1126-1136.
- Noremborg K., Strosznajder J. (1986) Modification of GABA and calcium uptake by lipids in synaptosomes from normoxic and ischemic brain. *Neurochem. Int.* 8: 59-66.
- Okajima Y., Nishi K. (1981) Analysis of inhibitory and excitatory actions of dopamine on chemoreceptor discharges of carotid body of cat in vivo. *Jap. J. Physiol.* 31: 695-704.
- Pokorski M., Strosznajder R. (1992) Phosphoinositides and signal transduction in the cat carotid body. In: *Control of breathing and its modelling perspective* (Eds. Y. Bondta et al.). Plenum Press, New York, p. 367-370.
- Pokorski M., Strosznajder R. (1993) PO₂-dependence of phospholipase C in the carotid body. In: *Neurobiology and cell physiology of chemoreception* (Eds. P.G. Data, H. Acker and S. Lahiri). Plenum Press, New York, p. 191-195.
- Shaw K., Montague W., Pallot D.J. (1989) Biochemical studies on the release of catecholamines from the rat carotid body in vitro. *Biochim. Biophys. Acta* 1013-1042.
- Shimizu T., Wolfe L.S. (1990) Arachidonic acid cascade and signal transduction. Short Review. *J. Neurochem.* 55: 1-15.
- Shinomura T., Mishima H., Matsushima S., Asaoka Y., Yoshida K., Oka M., Nishizuka Y. (1992) Degradation of phospholipids and protein kinase C activation for the control of neuronal functions. In: *Neurobiology of essential fatty acid. Advances in experimental medicine and biology* (Eds. N.G. Bazan, M.G. Murphy and G. Toffano). Vol. 318. Plenum Press, New York, p. 361-373.
- Siesjö B.K., Katsura K. (1992) Ischemic brain damage; Focus on lipids and lipid mediators. In: *Neurobiology of essential fatty acid. Advances in experimental medicine and biology* (Eds. N.G. Bazan, M.G. Murphy and G. Toffano). Vol. 318. Plenum Press, New York, p. 41-56.
- Simon M.F., Chap H., Douste-Blazy L. (1986) Selective inhibition of human platelet phospholipase A2 by buffering cytoplasmic calcium with the fluorescent indicator quin 2. Evidence for different calcium sensitivities of phospholipase A2 and C. *Biochim. Biophys. Acta* 875: 157-164.
- Strosznajder R.P. (1995) Ph.D. dissertation "Degradation of inositol phospholipids in mechanism of chemoreception in cat carotid bodies". Dept. of Neurophysiology, Medical Research Centre, P.A.S., Warsaw.
- Strosznajder J., Sun G.Y. (1981) Effects of acute hypoxia on incorporation of [¹⁴C] arachidonic acid into glycerolipids of rat brain. *Neurochem. Res.* 6: 767-774.
- Strosznajder J., Sun G.Y. (1982) Metabolism of fatty acids in brain membrane phosphoglycerides during normoxia and hypoxia. *Phospholipids in the nervous system, metabolism* (Eds. L. Horrocks et al.). Raven Press, New York, p. 111-122.
- Sun G.Y. (1992) Contributions to arachidonic acid release in mouse cerebrum by the phosphoinositide - phospholipase C and phospholipase A2 pathways. In: *Neurobiology of essential fatty acid. Advances in experimental medicine and biology* (Eds. N.G. Bazan, M.G. Murphy and G. Toffano). Vol. 318. Plenum Press, New York, p. 103-114.
- Urena J., Lopez-Lopez J., Gonzalez C., Lopez-Barneo J. (1989) Ionic currents in dispersed chemoreceptor cells of the mammalian carotid body. *J. Gen. Physiol.* 93: 979-999.
- Van der Vusse G.J., Van Bilsen M., Reneman R.S. (1994) Ischemia and reperfusion induced alteration in membrane

phospholipids. In: Cellular, biochemical and molecular aspects of reperfusion injury. Ann. N. Y. Acad. Sci. 723:1 p. 1-14.
Yoshida S., Ikeda M., Busto R., Sanisto M., Martinez E., Ginsberg M.D (1986) Cerebral phosphoinositide, triacylglycerol

and energy metabolism in reversible ischemia: origin and fate of free fatty acids. J. Neurochem. 47: 744-757.

Received 15 February 1996, accepted 23 February 1996

This paper is dedicated to Professor Stella Niemierko on the occasion of her 90th birthday, with esteem and admiration