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The influence of certain arachidonic acid pathway inhibitors on central nervous system catecholamine level in rats administered with ethanol

Wpływ wybranych metabolitów kwasu arachidonowego na zawartość katecholamin w centralnym układzie nerwowym szczurów po podaniu alkoholu etylowego

Ethanol has a depressive effect on CNS, similar to the general anaesthetics substances. However, the mechanism in which ethanol affects the CNS is not fully recognised yet. It is considered that the 5 to 10% concentrated ethanol inhibits the transmission in isolated peripheral neurones and muscles through selective dissociation of the synapses. Simultaneously, the enhanced epinephrine and norepinephrine secretion and high levels of catecholamine metabolites in the urine are observed. It is directly connected with the decrease in the amounts of catecholamines in the brain and brain stem (2).

The role of arachidonic acid metabolites in the catecholamines release and metabolism is also not well recognised. Presumably, prostaglandins can inhibit the release of norepinephrine (NE). It is well known that the cyclooxygenase inhibitor, i.e. indometacine, enhances the release of NE in CNS but it does not affect the NA uptake (8). Presynaptic inhibitory receptors for prostaglandins were identified on adrenergic neurones *in vitro* (7). Bradford's investigations show that indometacine and aspirin cause the decrease in the amounts of prostaglandins, allow the accumulation of free archidonic acid and diminish the potassium-stimulated release of (3H)dopamine (1). He also noticed that PGE2 inhibits, whereas PGF2 enhances the release of (3H)norepinephrine from the neurones. Wolf describes that catecholamines greatly activate the formation of

PGE2 alpha in the brain slices and homogenates, but not PGF2 level (16). On the other hand, dopamine is activated by PHS to electrophylic intermediators that covalently bind DNA, which can play the important role in dopaminergic neuronal degeneration (10).

Bibliography reviewed above shows that ethanol arachidonic acid metabolites can regulate the levels of catecholamines in CNS, so in present investigations the authors decided to examine the influence of different arachidonic acid inhibitors on the contents of dopamine (DA) and NA in the brains of the rats administered ethanol.

MATERIAL AND METHODS

Male Wistar rats (200-250) deprived of food but not water for 18-20 hrs before the experiment and held in the room temperature were divided into five groups. The first (I) control group (saline gropu) were the rats administered orally with 0.9% NaCl solution via stainless stomach tube (5 ml/kg b.w.). In the second group (II) (called ethanol group) rats received 35% ethyl alcohol (it was prepared as a fresh one before each experiment from 96% ethanol diluted in 0.9% NaCl to the required concentration) in the same way and equal amount as saline in the control group. In the third group (III) of rats, 5-lipooxygenase inhibitor – a compound AA 861 (Chem. Pharm. Inst. Tokyo Japan) was given intraperitoneally (*i.p.*) in the does 100 mg/kg of the b.w. The rats from the fourth (IV) group received 5-lipooxygenase (cyclooxygenase dual inhibitor – BW 755C (Wellcom Res. Lab. Beckenham, England) *i.p.* in the dose 100 mg/kg b.w. The fifth group of animals was administered with CGS 13080 – tromboxane TXA2 synthetase inhibitor (Ciba Geigy Summit USA) subcutaneously 1 mg/kg b.w.

Archidonic acid inhibitors were given 30 minutes before ethanol administration. The rats were killed by sharp blow on the head 2 hrs after ethanol or saline administration. Then brains were removed and immediately prepared in temperature above 0°C. The brains were homogenized in 0.2 M. CH_3COOH with the addition of 10 mg ascorbic acid and 50 mg Na_2EDTA on 1 g per wet tissue. Next 4M $HClO_4$ in the amount of 0.1 vol/vol was added to the homogenates and this mixture was centrifuged at 2000G in 4°C for 20 minutes. Supernatant was collected in the bacteriological tube and the sediment was twice dissolved with 3 ml 0.2 M CH_3COOH with the addition of 4 M. $HClO_4$. When the *pH* 6.5 in the supernatant was achieved, the fluid was placed in the glass absorption columns with specially activated AlO_2 acting as catecholamines absorbent. The elution of catecholamines from AlO_2 was performed with 5 ml 1 M CH_3COOH according to Górny and Flisiak's method (5). The amount of catecholamines in eluate was estimated according to L a v e r t y and T a y l o r method using the Farand's spectrofluorimetric model A (9). The results were analysed statistically by Student's test for unpaired data and expressed as arithmetical means ± standard deviations (X ± SD). $P< 0.05$ was considered statistically significant.

RESULTS

Ethanol given *per os* (5 ml/kg b.w.) caused a significant decrease in the level of dopamine ($p<0.001$) and norepinephrine ($p<0.001$) in the rats' brains in comparison to the control group administered with 0.9% NaCl. That treatment caused about the threefold decrease in the level of DA and the three-and--a-halffold decrease in NE level).

Table 1. The levels of dopamine (DA) [μ/g of tissue] and norepinephrine (NA) [μg/g tissue] in the whole brain after the administration of 0.9% NaCl, 35% ethanol, lipoxygenase inhibitor – AA-861, lipoxygenase/cyclooxygenase inhibitor-BW 775C and thromboxane TXA_2 inhibitor – CGS – 13080. The results are expressed as arithmetical means ± standard deviations (X ± SD) Student's t-test for unpaired data was used for statistical analysis. $p< 0.05$ was considered statistically significant: [★] – statistically significant difference between the saline and the ethanol group and also between the saline and each of the group pre-treated with AA pathway inhibitor, [*] – statistically significant difference between the ethanol group and each of the group pre-treated with AA pathway inhibitor,★ $p<0.05$, ★★ $p<0.01$, ★★★ $p<0.001$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$

	0.9% NaCl	35% ethanol	AA-861	BW 755C	CGS-13080
DA x ± SD	0.556 ± 0.039	0.190 ± 0.037 ★★★	0.684 ± 0.162 ★★/***	0.514 ± 0.114 ★/***	0.879 ± 0.474 ★★★/***
NA x ± SD	0.546 ± 0.089	0.155 ± 0.051 ★★★	0.180 ± 0.044 ★★★/*	0.127 ± 0.037 ★★★/*	0.067 ± 0.037 ★★★/***

The 5-lipoxygenase inhibitor AA 861 given parenterally 30 minutes before the alcohol administration caused a statistically significant rise in the DA level in relation to the saline group ($p<0.01$) and also to the rats administered with ethanol ($p<0.001$). AA 861 decreased the content of NE in the rats CUN three times compared with the saline given group ($p<0.001$) but increased NA level in comparison with the ethanol group ($p<0.05$).

The 5-lipooxygenase/cyclooxygenase dual inhibitor BW 755 C given parenterally before the ethanol administration caused the decrease in the DA content in relation to the saline administered rats ($p<0.05$), but the level of DA decreased about three times in comparison with the ethanol given animal. After BW 775C the level NA was diminished over four times in relation to the saline group ($p<0.001$) but it was higher in comparison with the ethanol administered rats ($p<0.05$).

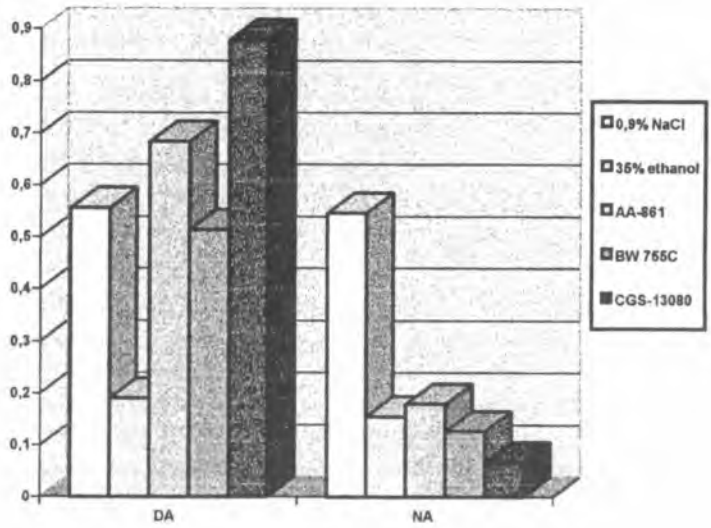

Fig. 1. Dopamine and norepinephrine levels [mg/g tissue] in brains of rats administered with 0.9% NaCl or 35% ethanol and pre-treated with AA-861, BW-755C and CGS-13080

The level of DA in the rats brains increased significantly after the treatment with TXA2 synthetase inhibitor – CGS 13080 in comparison with both groups – saline ($p<0.001$) and ethanol administered one ($p<0.001$). Adversely, CGS 13080 caused nearly eightfold decrease in NE level in relation to the saline administered rats ($p<0.001$) and over twofold decrease in NA in relation to the ethanol given rats ($p<0.01$).

DISCUSSION

Our results show that the acute ethanol exposure causes the decrease in norepinephrine content in the rats brains. These results are similar to B o w m a n's investigations (2). He observed that ethanol enhances the release of NE from neurones and it is closely connected with the decrease in NE content in the brain. T a n a k a (13) noticed that ethanol by itself increased NE release in a hypothalamus.

Probably the NE release is at least partially dependent on the level of prostaglandins in CNS. The cyclooxygenase inhibitor – indometacine, causes the increase in the release of NE and does not act on NA uptake (8). Kenneth, Eino and Rossi investigations, who identified the presynaptic inhibiting receptors for prostaglandins in the adrenergic neurones suggest that PGs inhibit the release of

NE from the neurones (7). Here, it is important that ethanol does not inhibit 15 hydroprostaglandin dehydrogenase which metabolizes PGE2 to inactivated metabolite – 15-keto-PGE2 and in this way does not change PGs (14,15).

One the other hand, it is known that ethanol acts as the inhibitor of two important enzymes of arachidonic acid pathawy: phospholipase A2 and cyclooxygenase. N a v a m a n i et al. (11) investigating glutamate-evokes a Ca 2+ dependent relase of arachidonic acid from eultured neurones observed that the acute ethanol administration (100mM, 15 min) inhibited the release of AA from the neurones. F e n n et al. (4) also maintain that ethanol can inhibit Ca2+ activated phospholipase A2 activity in superfused rats brains. R e t t o r i and others (12) found that ethanol inhibits the conversion of labelled AA to PGE2, so it can inhibit the cyclooxygenase directly or indirectly.

So, after the acute ethanol exposure the level of PGs in the brains declines as a result of the increase in the NE release from the neurones.

R e t t o r i's, C a n t e r o s' and W i n e y's investigations concerning connections between the ethylic alcohol, catecholamines and PGs synthesis found that NE indirectly, via NO, enhances the production of PGs in the hypothalamus and ethanol inhibits PG production (3,6,12).

In our investigations the 5-lipooxygenase inhibitor (AA 861) caused the increase in NE level in rat brains in relation to the ethanol administered animals. In the rats pretreated with AA 861 before the alcohol, the whole free arachidonic acid could be conversed to PGs. Consequently, the level of PGs was higher than in the group administered only with ethanol, so it seemed that PGs inhibiting NE release caused the NE content in the brain increased.

BW 755C-5-lipooxygenase-cyclooxygenase dual inhibitor intensified the ethanol inhibiting action on cyclooxygenase and also blocked 5-lipooxygenase. Hence free AA could not be conversed to both PGs and leucotriens. The level of PGs decreased more than in the group administered only with ethanol and in the group pre-treated with AA 861 before ethanol. Finally the level of NE in the brain was lower in comparison with those groups.

According to bibliography and in accordance with the results of our earlier investigations we have a good reason to suggest that in the groups of rats pretreated with the thromboxane inhibitor, the level of NE in the brain will increase in comparison with the groups pretreated with other inhibitors since theoretically, all endoperoxides can be converted to PGs. However, we found that the content of NE decreased and the DA content increased in relation to the saline, ethanol and other studied groups.

Our results show that the acute ethanol exposure causes the decrease in the DA level in rat brains similarly to the decrease in NE content.

T a n a k a (13) noticed that in ethanol administered rats the DA release from the nucleus accumbens increased so its content decreased. In the accessible literature the authors have found fewer reports investigating the connections between dopamine and the metabolites of arachidonic acid pathway.

The experiments show that DA content in the 5-lipooxygenase inhibitor pre-treated group is significantly higher than in the saline as well as ethanol administered groups. After 5-lipooxygenase/cyclooxygenase dual inhibitor, the level of DA in the rat brains decreased in comparison with the saline administered group and increased in relation to the ethanol group, as well as in the 5-lipooxygenase inhibitor pre-treated group. In the TXA2 pre-treated group the content of dopamine was higher than in the saline administered group. To explain the influence of AA pathway inhibitors on the activity of catecholamine pathway enzymes further investigations are needed.

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STRESZCZENIE

Celem naszej pracy było zbadanie wpływu inhibitorów przemian kwasu arachidonowego na zawartość dopaminy i norepinefryny w mózgu szczurów po podaniu alkoholu etylowego. Wykazaliśmy, że etanol powoduje spadek zawartości norepinefryny i dopaminy w mózgu szczurów w porównaniu z grupą otrzymującą sól fizjologiczną. Inhibitor 5-lipooksygenazy – AA861 zwiększał zawartość dopaminy w porównaniu z grupami otrzymującymi sól fizjologiczną oraz etanol. Jednocześnie poziom adrenaliny obniżył się w stosunku do grupy kontrolnej, a wzrósł w stosunku do grupy, w której podawano etanol. Podwójny inhibitor 5-lipooksygenazy/cyklooksygenazy – BW755C w sposób znamienny podnosił zawartość dopaminy w porównaniu z grupami, które otrzymywały sól oraz etanol i powodował spadek poziomu norepinefryny w stosunku do grupy kontrolnej i otrzymującej jedynie etanol. W grupie szczurów, które otrzymywały inhibitor syntetazy TXA2 – CGS1308, zaobserwowaliśmy wzrost poziomu dopaminy w stosunku do grupy kontrolnej i etanolowej. CGS1308 powodował spadek poziomu norepinefryny w porównaniu z grupą kontrolną i etanolową.