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BRAIN BIOGENIC AMINE LEVELS AFTER METHANOL ADMINISTRATION : POSSIBLE MECHANISM OF ACTION ON CENTRAL MONOAMINERGIC NEURONS IN DISCRETE AREAS OF BRAIN IN WISTAR RAT

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Abstract : Alterations in the steady state level of rat brain biogenic amines - dopamine, nor-epinephrine, epinephrine, serotonin and 5-hydroxy indole acetic acid, in response to intraperitoneal administration of methanol (3g/kg b.w.) were studied in discrete areas of the rat brain. The monoamine changes induced by methanol were quite different from those induced by ethanol consumption. They were also region-specific; hypothalamus being more vulnerable for methanol-induced monoamine changes. The effects produced by methanol were correlated with the blood and brain level of methanol at the given time, suggesting that the effects were dependent upon the local concentration of methanol in different brain regions. Acidosis induced by ammonium chloride and sodium formate administration did not alter the monoamine levels and therefore, the effects of methanol were not possibly due to acidosis. Blocking or delaying the metabolism of methanol either by 4-Methyl Pyrazole and 3-Amino·1,2,4-Triazole or by simultaneous administration of ethanol resulted in the potentiation of methanol effect. Therefore, it was concluded that methanol induced changes in brain biogenic amines were due to methanol *per se* and not due to metabolic end products *viz.* formaldehyde or formic acid.

Key words : methanol monoamines 4-methyl pyrazole amino triazole

INTRODUCTION

Several chemicals, drugs and stressors are known to alter the function and/or the metabolism of some putative neuro-transmitters in the central nervous system (2, 3). Biogenic amines such as dopamine (DA), nor-epinephrine (NE), epinephrine (E) and serotonin (5–HT) are of particular interest, since changes in the levels of these amines may lead to alteration in the behaviour pattern. Without question, ethanol is sociologically the most significant drug of abuse besides the opioids. The role of ethanol in altering the steady state level and/or the turnover rate of these brain biogenic amines were studied extensively by several workers in the past (8, 12). Brain monoamines play a very significant

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role in the neuro-chemical changes leading to physical dependence of alcohol (7). Unlike ethanol, methanol produces severe metabolic toxicity in man even when small volume is consumed. The toxicity of methanol is unique in the sense that it produces severe ocular dysfunction leading to blindness in several cases (5, 13, 14, 17). Recently we have demonstrated that methanol also interferes with the steady state level and/or the turn over rate of brain monoamines (9). As a step towards identification of the differences in the effects produced by the two different alcohols viz. ethanol and methanol on the brain monoaminergic neurons, it was considered necessary to understand the mode of action of methanol. Hence the present work using the Wistar rat as a the experimental animal model was initiated.

METHODS

Experimental animals were healthy adult male Wistar rats weighing approximately 150–230 g. All the animals were maintained under standard laboratory conditions. They were housed 5 per cage (29cm × 22cm × 14 cm) and were fed standard rat feed pellets ('Gold Mohur', supplied by M/s Hindustan Lever Ltd., India) *ad libitum*. In order to eliminate possible variations in brain biogenic amine levels due to circadian rhythm, experiments were always conducted between 8 am and 11 am. Animals were deprived of food for 12–14 hr prior to experimentation. The animals were divided into the following groups for the present study. Group-I : The animals in this group were given methanol (3g/kg, bw) as 30% solution wt/vol in saline intraperitoneally (ip). Some animals (n=9) were sacrificed at the end of 30 min and the rest (n=9) at 60 min following methanol treatment. To Group-II, Ammonium chloride (100 mg/kg, bw) was administered intra-peritoneally. To Group-III, sodium formate was given (100 mg/kg, bw-ip). Animals in group II & III were sacrificed at the end of 30 and 60 min respectively in two sub-groups (n=9). To Group-IV, ethanol (3 g/kg, bw) and methanol (3 g/kg, bw) were given simultaneously and sacrificed at the end of 30 and 60 minutes in two sub-groups (n=7). In all these groups, appropriate number of control animals were maintained (n=13) which received equal volume of normal saline (0.9% NaCl). To Group-V, 4-Methyl Pyrazol (50 mg/kg, bw) and 3-Amino-1, 2, 4-Triazole (1 g/kg, bw) were administered one hour prior to administration of methanol (3 g/kg, bw). Animals receiving the drugs alone served as appropriate controls (n=7). All the animals were sacrificed at 30 and 60 min intervals.

Animals were sacrificed by decapitation after various treatments in the different groups. The brains were removed immediately after sacrifice and were dissected into seven discrete regions viz. hypothalamus, hippocampus, striatum, cerebellum, midbrain, pons-medulla and cerebral cortex as described by Glowinski and Iversen (6). Tissue pieces were weighed immediately to avoid drying and homogenised in acidified n-butanol for assay of dopamine, norepinephrine, epinephrine, serotonin and 5-hydroxy indole acetic acid by fluorimetric method by the method of Kari *et al.*, (10), using a Hitachi model 650-10M fluorescence spectrophotometer. The recovery of each of the monoamines in this method ranged from 65% to 85% and the assays were reproducible within 5%. All the data obtained were corrected for appropriate recoveries.

Blood was collected directly from the decapitated body and blood methanol was estimated by Type-B procedure (1) using an Eppendorff-6120 photometer. Methanol levels in the same procedure. The type-B procedure was accurate enough to detect a blood methanol level of as low as 10mg% and reproducible within 3mg%. Venous blood pH, PCO_2 and HCO_3 were monitored using an Acid Base Laboratory (ABL-3 model, Copenhagen) to check the degree of acidosis in the group I, II & III animals. Data were statistically analysed using Student's 't' Test.

RESULTS AND DISCUSSION

In the hypothalamus, there was an increase in the steady state level of dopamine, 5-HT and 5-HIAA level of 60 minutes after methanol administration, whereas, NE and E levels were showing a decrease in their concentration. In the striatum, the DA and NE levels as well as 5-HT and 5-HIAA levels were reduced significantly whereas there was no change observed in epinephrine level. In the midbrain, NE and E levels were found to be reduced whereas 5-HT and 5-HIAA levels were increased. Dopamine level did not show any significant change. In the pons-medulla, NE level was reduced and 5-HIAA level increased. The other monoamines (DA, E and 5-HT) did not show any significant change in their concentrations. In the cerebral cortex, NE and 5-HIAA levels increased. The levels of DA E and 5-HT did not show any significant change. Hippocampus and cerebellum did not show significant change in any of these monoamine levels after methanol administration. Serotonin level was increased in hypothalamus, midbrain and cerebral cortex with corresponding increase in the level of its metabolite, 5-HIAA, indicating an increased turn over rate of 5-HT in these areas after methanol administration (Figs. 1, 2, 3, 4 & 5).

It was observed in our preliminary studies that when blood methanol level was followed up till 240 minutes after methanol administration. the blood methanol level reached the maximum at the end of 60 minutes and hence it could be correlated with the monoamine changes at 60 min after methanol administration. Therefore, although similar changes of lesser magnitude were observed in the brain biogenic amine levels at differet time intervals after various treatments, only the 60 min data were taken into consideration for highlighting the findings in this paper. Methanol level was found to be lower in all the brain areas as compared to blood methanol level (300 mg%). In the brain, the maximum concentration of methanol (280 mg%) was detected in the hypothalamus; all the other areas contained more or less (150–200 mg%) identical concentration of methanol. The location of a number of 'extra blood-brain barrier' areas (4) in this region (such as organum vasculosum, lamina terminalis, tuberal region, sub-fornical organ etc.,) appears to be in favour of increased capillary permeability of this part of the brain, leading to a higher methanol level in the hypothalamus. Therefore, it could be presumed that the effect of methanol on brain monoamines was dependent upon the local regional concentratinn of methanol.

To elucidate the role of acidosis in induction of these changes, metabolic acidosis was induced by administration of ammonium chloride (group-II) and sodium formate (group-III). Inspite of producing acidosis as evidenced by a fall in HCO_3 content and pH in the blood, no changes were observed to occur in monoamine levels in any of the areas examined (Table-I). This is obviously due to the fact that the H^+ ions do not cross the blood brain barrier with ease. The purpose of administration of ethanol and methanol simultaneously (group-IV) is to retard the metabolism of methanol, as ethanol competes with methanol for the common enzyme-alcohol dehydrogenase (15, 16). Our results from group-IV animals have shown that blood and brain level of methanol have been substantially elevated and sustained. At the same time, the pattern of monoamine changes observed was not very much dissimilar to methanol alone treated animals (group-I). But quantitatively, the changes were much more pronounced than the changes produced by methanol alone (Fig. 1, 2). Thus, intra-cellular concentration of methanol may be a determining factor in bringing about the monoamine changes in various discrete areas of rat brain.

Pre-treatment with 4-Methyl Pyrazole and Amino Triazole delayed considerably the elimination of methanol from the circulation, so that the brain methanol was also increased. With the occurrence

TABLE I : *Brain Monoamine levels in Discrete Areas.*

Experimental animals were given either ammonium chloride (100 mg/kg) or sodium formate (100 mg/kg). n=9 in each group. Control animals were given equal volume of normal saline (n=13). All the animals were sacrificed at the end of 60 min. Monoamine values are expressed in ng/g brain tissue as Mean±SE.

Area of Brain	*Treatment*	*Dopamine*	*Norepinephrine*	*Epinephrine*	*Serotonin*	*5-HIAA*
Hypothalamus	Saline	693±56	1709±101	199±33	1020±71	972±82
	Ammonium chloride	723±54	1853±116	197±37	1064±108	1033±102
	Sodium formate	722±91	1720±163	181±47	1004±100	928±120
Hippocampus	Saline	126±24	322±38	100±26	503±48	355±49
	Ammonium chloride	130±29	334±55	114±30	480±56	381±57
	Sodium formate	150±35	354±64	119±29	524±58	337±64
Striatum	Saline	5214±550	475±54	205±39	684±49	581±45
	Ammonium chloride	5044±403	492±74	223±45	693±61	599±58
	Sodium formate	5427±740	482±76	230±64	751±75	624±83
Cerebellum	Saline	91±25	296±41	127±35	124±27	354±47
	Ammonium chloride	100±32	267±51	165±38	144±21	339±60
	Sodium formate	106±38	280±61	149±27	137±33	403±47
Mid Brain	Saline	308±42	507±50	326±25	746±49	1021±52
	Ammonium chloride	301±57	508±72	322±31	720±70	995±67
	Sodium formate	326±77	518±75	416±55	763±124	1002±89
Pons-Medulla	Saline	190±29	630±43	298±39	617±49	617±48
	Ammonium chloride	155±34	730±81	417±51	737±39	696±52
	Sodium formate	199±47	777±111	438±59	730±106	650±90
Cerebral Cortex	Saline	754±55	293±43	128±24	424±37	581±38
	Ammonium chloride	736±54	313±59	145±29	468±43	600±53
	Sodium formate	743±81	309±70	176±36	461±68	616±75

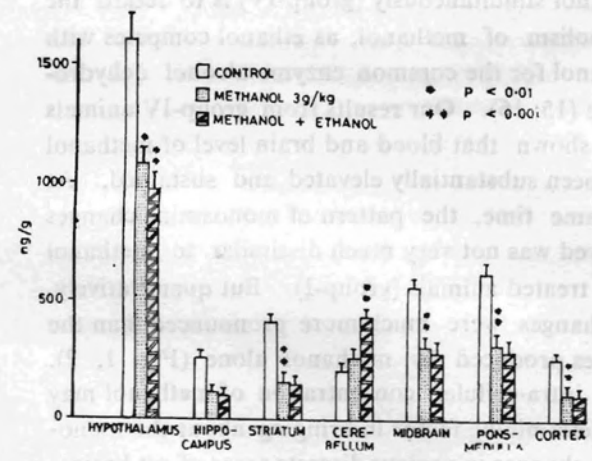

Fig. 1 : Nor-Epinephrine level in Discrete Brain Areas

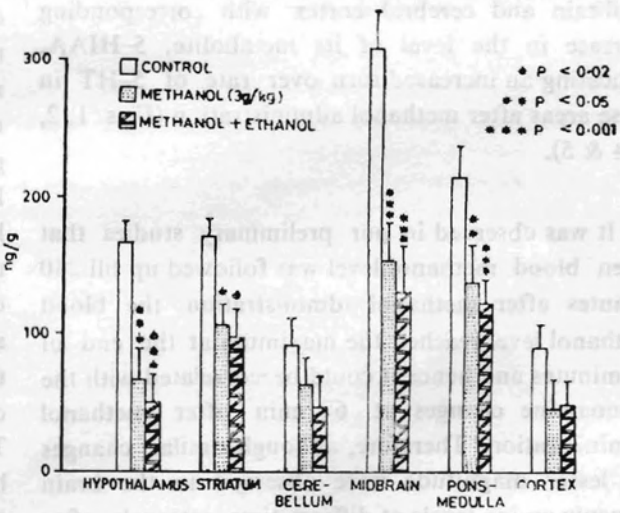

Fig. 2 : *Epinephrine Level in Discrete Brain Areas.*

Methanol was given at a dose of 3 g/kg b. w. Ethanol+Methanol were given simultaneously at a dose of 3 g/kg, bw each (n=7). Control animals were given normal saline of equal volume (n=13). All the animals were sacrificed 60 minutes after their respective treatments.

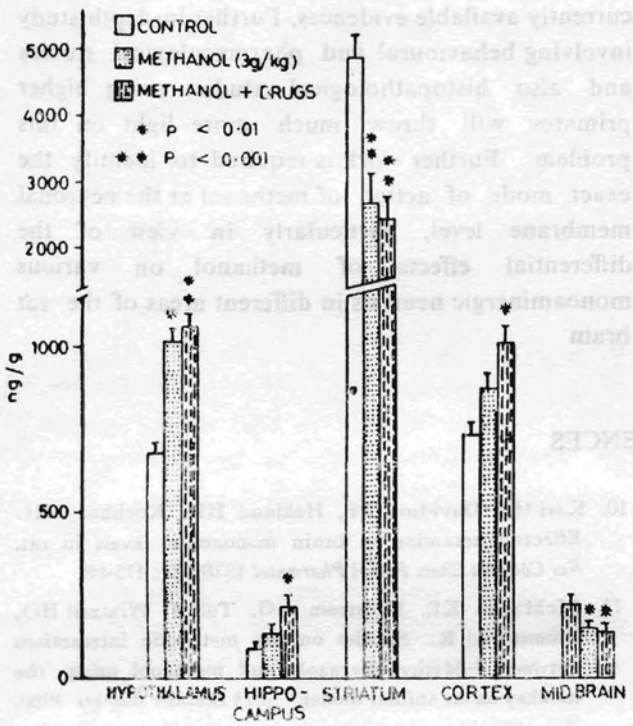

Fig. 3 : Dopamine level in Brain Areas

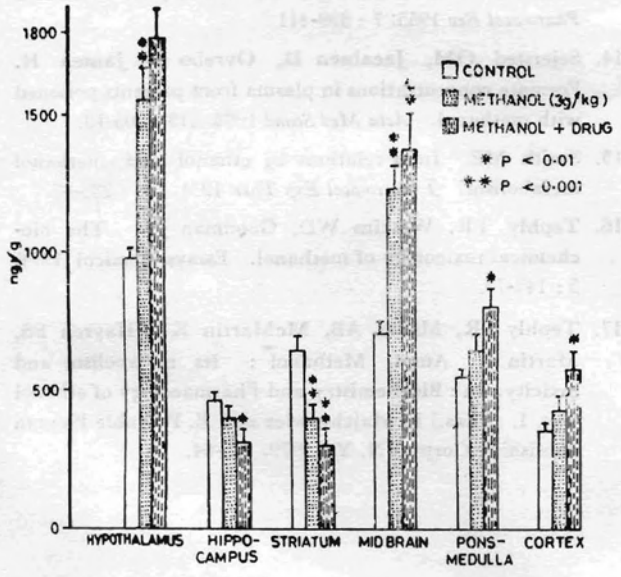

Fig. 4 : 5-HT level in Brain Areas

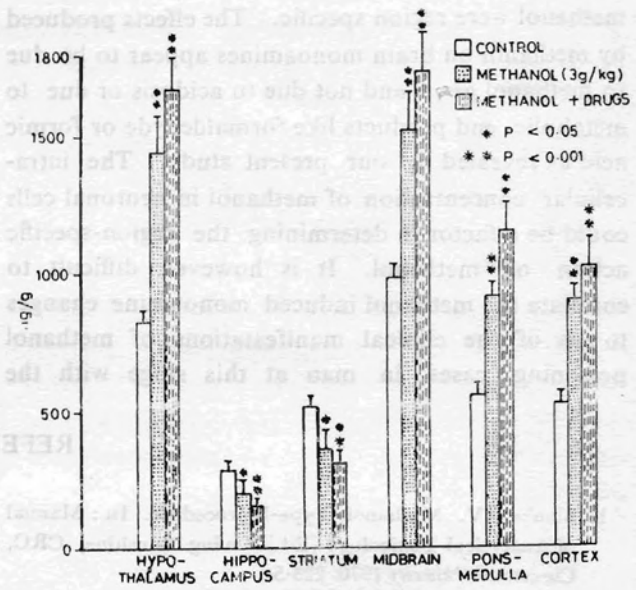

Fig 5 : *5 HIAA level in Discrete Brain Areas*

Methanol was given at a dose of 3 g/kg b. w. as 30% solution wt vol (n=7). Methanol + Drugs : 4-Methyl Pyrazole (50 mg/kg) and 3-Amino-1,2,4-Triazole (1 g/kg) were administered one hour prior to administration of methanol (3 g/kg) n=7. All the animals were sacrificed at the end of 60 minutes. Control animals were given equal volume of drugs alone (n=7).

of these changes in methanol level, the changes observed in brain monoamines were also more pronounced. This indicates that the greater the methanol concentration in a given area of the brain, the greater will be the effect of methanol. Since the breakdown of methanol is completely inhibited by the enzyme blockers, the changes induced by methanol could not be due to the metabolic end products of methanol. However, there is no unequivocal evidence available in the literature to confirm the presence of aleohol dehydrogenase or catalase in the rat brain tissue to account for the metabolism of methanol *in situ* to any considerable extent. The enzyme blockers used in this study could only have blocked the metabolism of methanol in the liver and other peripheral tissues if any.

In conclusion, it could be presumed that the monoamine changes in rat brain induced by

methanol were region specific. The effects produced by methanol on brain monoamines appear to be due to methanol *per se* and not due to acidosis or due to metabolic end products like formaldehyde or formic acid as revealed by our present study. The intracellular concentration of methanol in neuronal cells could be a factor in determining the region-specific action of methenol. It is however, difficult to correlate the methanol induced monoamine changes to any of the clinical manifestations of methanol poisoning cases in man at this stage with the currently available evidences. Further in-depth study involving behavioural and pharmacological studies and also histopathological studies using higher primates will throw much more light on this problem. Further work is required to identify the exact mode of action of methanol at the neuronal membrane level, particularly in view of the differential effects of methanol on various monoaminergic neurons in different areas of the rat brain

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