Ann. Ist. Super. Sanità
Vol. 26, N. 1 (1990), pp. 95-98

VOLUNTARY ALCOHOL DRINKING INCREASES BRAIN DOPAMINE METABOLISM IN RATS

G. COLOMBO *(a)*, F. FADDA *(a)*, G.L. GESSA *(a)*, A. MAISKY *(b)* and E. MOSCA *(a)*

(a) Dipartimento di Neuroscienze «Bernard B. Brodie», Università degli Studi, Cagliari, Italy
(b) Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow.

Summary. - *The effect of ethanol on dopamine (DA) metabolism in two selectively bred lines of rats, one alcohol-preferring (sP) and the other - non preferring (sNP), was studied. Ethanol administration (2 g/kg* per os*) produced in the two lines of rats a decrease of DA content and an increased concentration of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the caudate nucleus, olfactory tubercle and medial prefrontal cortex in both lines, but the effect was significantly greater in sP than in sNP rats. Moreover, in sP rats, the voluntary consumption of ethanol increased DOPAC and HVA levels in the above areas. In these animals, DOPAC and HVA accumulation was associated with a small depletion in DA content, suggesting that ethanol releases DA from stores.*

Riassunto (Aumento del metabolismo della dopamina cerebrale nel ratto indotto dall'assunzione di alcool). - *È stato studiato l'effetto dell'alcool sul metabolismo della dopamina (DA) cerebrale utilizzando ratti alcool-preferenti (sP) e -non preferenti (sNP), appartenenti a due linee di ratti selezionati geneticamente nel nostro laboratorio. La somministrazione di etanolo (2 g/kg* per os*) ha prodotto nelle due linee di ratti una diminuzione del contenuto di DA e un incremento dei suoi metaboliti, l'acido 3,4-di-idrossifenilacetico (DOPAC) e l'acido omovanillico (HVA), nel nucleo caudato, tubercolo olfattorio e corteccia mediale prefrontale. Tale effetto è stato significativamente più marcato nei ratti sP che nei ratti sNP. Inoltre nei ratti sP il consumo volontario di etanolo ha determinato un incremento del DOPAC e dell'HVA nelle aree su citate. In questi animali l'accumulo del DOPAC e dell'HVA era associato ad una leggera caduta dei livelli di DA, suggerendo che l'etanolo libera DA dalle riserve.*

Introduction

Considerable evidence suggests that central dopamine (DA) neurons play an important role in mediating the reinforcement properties of drugs of abuse such as amphetamine [1, 2], cocaine [3], morphine [4, 5], heroin [6] and even natural reinforcers such as food and water [7, 8]. Consistently, in our laboratory we have recently found that ethanol administration stimulates the firing rate of brain DA containing neurons both in the *substantia nigra* and in the ventral tegmental area (A10) [10], which project to the basal ganglia and to limbic areas, respectively. Moreover, we found that A10 DA neurons are more sensitive to ethanol than DA neurons located in the *pars compacta* of the *substantia nigra* (A9).

Recent findings by Imperato and Di Chiara have shown that small doses of ethanol preferentially stimulate DA release in the *nucleus accumbens* rather than in the caudate nucleus in freely moving rats [11, 12].

In order to further clarify the neurochemical correlates of alcohol seeking behaviour, we studied the effect of ethanol on brain DA metabolism in two lines of rats, one of which in a free choice condition preferentially consumes 10% (w/v) ethanol solution and the other one almost totally avoids it.

Moreover, we studied whether spontaneous ethanol drinking had any effect on brain DA metabolism.

Materials and methods

Two lines of rats, one alcohol preferring and the other alcohol-non preferring, were developed in our laboratory through selective breeding from Wistar

rats (Charles River, Como, Italy) according to the method of Lumeng *et al.* [13]. To distinguish our newly developed lines from those developed in Li's laboratory [14] at Indianapolis we have termed our rats by the symbols sP and sNP, meaning Sardinian alcohol-preferring and non-preferring. In the present study we have utilized the male rats from the 13th generation. Starting from the 30th day of age, rats were individually housed in a room at 22°C and 60% relative humidity, having reversed light-dark cycle with light phase from 20.00 to 8.00 and dim red light from 8.00 to 20.00 hours. Rats had free access to standard laboratory food, tap water and 10% ethanol solution in water (w/v). Fluids were offered according to the two bottle free choice procedure [15]. Within 3 weeks of isolation, rats showed a stable baseline of ethanol or water consumption.

Experiment a) ethanol administration

Two groups of 24 rats each, one sP and the other sNP, weighing 200-220 g, were used. The animals were approximately 60 days old and consumed 7.6 ± 0.6 and 1.2 ± 0.3 g/kg of ethanol daily, respectively.

Ethanol was withdrawn for 48 h and then half of the rats of each group received by gavage 2 g/kg ethanol as 20% solution in water, whereas the other half received by gavage the corresponding volume of water.

Experiment b) voluntary ethanol ingestion

One group of 24 sP rats, of approximately 60 days of age, was habituated to drink its daily fluid in a restricted period of 2 h, by presenting the two bottles, one containing water and the other 10% ethanol solution, at 10.00 and removing them at 12.00, during the dark phase. Food was always available *at libitum*. Within 2 weeks of habituation animals reached a stable baseline intake of about 90 ml/kg fluid and of 5.1 ± 0.4 g/kg ethanol within the two-hour period (Fig. 1). On the 15th day of such schedule animals were divided in two subgroups: to the first one the two bottles were presented as usually, to the other only water was offered instead. Animals were sacrificed 60 min after fluid presentation, during which time they had drunk most of their daily fluid intake.

For brain analyses caudate nucleus, medial prefrontal cortex (MPFC) and olfactory tubercle were dissected on ice and stored at -30 °C until analyzed. The content of DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) was assayed using reverse phase high pressure liquid chromatography (HPLC) coupled with electrochemical detection as previously reported [16]. In brief, tissue samples were homogenized in 0.1 N $HClO_4$ (1:25, weight:volume) containing 3,4-dihydroxybenzylamine (DHBA) as internal reference standard. Following

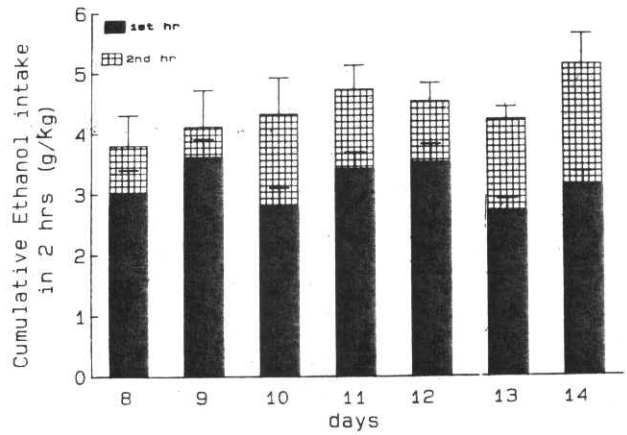

Fig. 1. - Voluntary ethanol ingestion. Rats were habituated to drink their daily fluid in a period of 2 h by presenting the two bottles, one containing H_2O and the other 10% ethanol solution. After 14 days of habituation, animals consumed about 90 ml/kg fluid and 5.1 ± 0.4 g/kg ethanol solution in the two-hour period. Solid columns = amount of ethanol consumed within the 1st h.

centrifugation at 10,000 × *g* for 20 min, the supernatant was filtered in a Millipore apparatus. DA, DOPAC and HVA were measured in the supernatant applied directly to the HPLC system.

Blood ethanol concentrations were determined by gas chromatography procedure, in blood samples collected from trunk blood at the time of killing.

Statistical analyses were performed by analysis of variance.

Results

Ethanol administration to sP and sNP rats

As Fig. 2 shows, ethanol administration to sNP rats significantly increased DOPAC and HVA levels both in the caudate nucleus and in the olfactory tubercle and produced a modest decrease in DA content in these areas. In these rats ethanol failed to modify DA metabolism in the MPFC.

However, in sP rats ethanol influenced DA metabolism to a greater degree than in sNP ones. In fact in sP rats, ethanol increased DOPAC by 80, 88 and 30% in the caudate nucleus, olfactory tubercle and MPFC, respectively. In the same areas, HVA concentration was enhanced by 64, 72 and 25%, respectively. *Vice versa*, DA concentration was decreased by 30, 35 and 24%, respectively.

Voluntary ethanol ingestion by sP rats

The effect of ethanol, voluntarily consumed by sP rats, on DA metabolism was studied. As specified in the methods, these animals were sacrificed one hour after presentation of the ethanol solution. Within this period they drank an average of 3.1 ± 0.7 g/kg of ethanol (Fig. 1). Fig. 3 shows that the spontaneous ethanol ingestion increased DOPAC levels by 38, 75

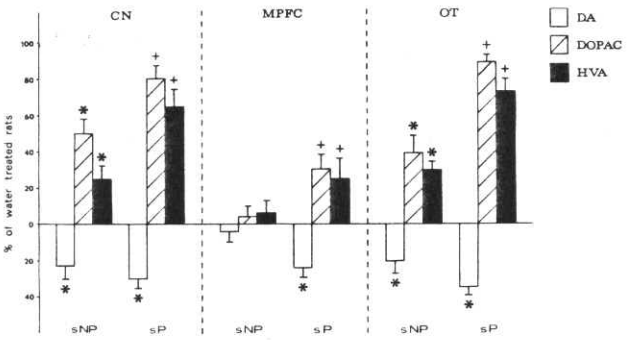

Fig. 2. - Effect of ethanol administration (2 g/kg) on DA, DOPAC and HVA concentrations in different brain areas in alcohol-preferring (sP) and alcohol non-preferring (sNP) rats. Ethanol (20% solution in water, w/v) or water was given orally by gavage. Rats were sacrificed 60 min after treatment. Results are the mean ± SEM of data of three independent experiments, in each of which 4 rats per group were used (no. = 12). Values are expressed as percentage of values obtained from water-treated rats. Values of water-treated sP and sNP rats were not statistically different, therefore they were pooled. Values for DA in the caudate nucleus (CN), medial prefrontal cortex (MPFC) and olfactory tubercle (OT) were (ng/g wet tissue): 11,815±702, 81±4 and 7326±39, respectively. In the same areas DOPAC concentrations were 1436±86, 33±2 and 1794±88; HVA concentrations were 964±51, 25±4 and 582±41, respectively.
* $p < 0.01$ with respect to water-treated rats; + $p < 0.05$ with respect to sNP rats receiving ethanol.

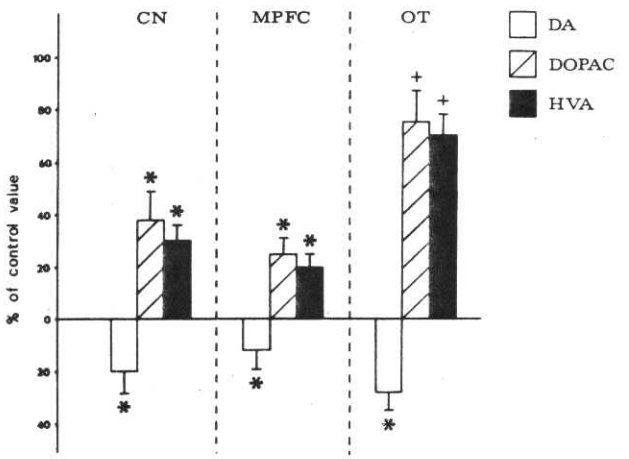

Fig. 3. - Effect of voluntary ethanol consumption on DA, DOPAC and HVA levels in different brain areas. sP rats were adapted to drink their daily fluid, i.e. 10% ethanol or water solution, in a restricted time interval of 2 h (see "Methods"). They were sacrificed 60 min after fluid presentation. Within this time period they consumed an average of 3.1±0.7 g ethanol/kg b.w. Values are means ± SEM obtained from three experiments each of which comprised 4 animals per group (no. = 12). Results are expressed as percentage of values obtained from sP rats which drank water instead of ethanol solution. In water-drinking animals DA values (ng/g wet tissue) in the caudate nucleus (CN), medial prefrontal cortex (MPFC) and olfactory tubercle (OT) were 11,602±724, 84±3 and 6925±189, respectively. In the same areas DOPAC values were 1506±38, 31±2 and 1602±34; HVA values were 856±49, 26±3 and 732±54, respectively.
* $p < 0.01$; + $p < 0.001$ with respect to water-treated rats.

and 25% in the caudate nucleus, olfactory tubercle and MPFC, respectively. In the same areas, HVA content was increased by 30, 70 and 20%, respectively. DA concentration was decreased by 20, 28 and 12%, respectively.

Blood samples collected from trunk blood, 60 min after treatments indicated BACs of 195±16 and 219±14 mg% in sP and sNP rats, respectively, receiving ethanol by gavage, and 211±56 mg% in sP rats after voluntary ethanol consumption.

Discussion

The present results confirm previous observations that ethanol administration increases brain DA metabolism [17-19]. However, the two major findings in the present work are that ethanol modifies brain DA metabolism to a greater degree in sP than in sNP rats and that voluntary ethanol ingestion is associated with a marked increase in DA metabolism in different brain areas.

The different effect of ethanol in sP and sNP rats does not depend on pharmacokinetic differences, being the BACs in the 2 lines of animals not statistically different.

On the other hand, the greater effectiveness of ethanol in sP rats might reflect either a genetic trait, possibly linked to ethanol preference, or it might be the consequence of the fact that sP rats had consumed ethanol chronically prior to the ethanol challenge [15]. If the second hypothesis is correct, our finding might imply that chronic ethanol consumption produces a sensitization of DA neurons to the action of ethanol. In order to clarify this problem we are presently studying the effect of ethanol on DA metabolism in rats of sP and sNP strains that never experienced ethanol beforehand.

Voluntary ethanol ingestion produced similar change in brain DA metabolism as after the oral administration of the drug by gavage. Since the dose voluntarily ingested by our rats was within the range that may be consumed by human alcoholics it is likely that our findings have clinical relevance.

As to the mechanism by which ethanol increases DOPAC and HVA levels, it is likely that this effect is the consequence of ethanol-induced release of DA from nerve terminals and of the enhanced retrieval and metabolism of the amine [17]. In agreement with such a proposition is the finding that DOPAC and HVA accumulation was associated with a modest depletion of DA content, also suggesting that DA synthesis rate is insufficient to cope with ethanol-induced release and degradation of DA. Accordingly, chronic ethanol administration has been shown to produce tolerance to its stimulant effect on DA synthesis [15].

The dose of ethanol administered to our rats was inappropriate for demonstrating a preferential action

on limbic areas versus the caudate nucleus, but the dose was chosen to be similar to that which rats obtain through voluntary ethanol consumption.

Considering that dopaminergic neurons seem to play an important role in brain reward mechanism [20], the finding that voluntary ethanol consumption increases DA metabolism and that such effect persists after chronic ethanol consumption might be relevant in explaining the biological basis of ethanol dependence.

Acknowledgements

This work was partially supported by CT Laboratories, Sanremo and by a Grant from CNR 87.01418.04.

REFERENCES

1. LE MOAL, M., STINUS, L. & SIMON, H. 1979. Increased sensitivity to (+)-amphetamine self-administration by rats following mesocorticolimbic dopamine neurons destruction. *Nature* **280**: 156-158.

2. LYNESS, W.H., FRIEDLE, N.M. & MOORE, K.E. 1979. Destruction of dopaminergic nerve terminals in *nucleus accumbens*: effect on D-amphetamine self-administration. *Pharmacol. Biochem. Behav.* **11**: 553-556.

3. ROBERTS, D.C.S. & KOOB, G.F. 1983. Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. *Pharmacol. Biochem. Behav.* **17**: 901-904.

4. GYSLING, K. & WANG, R.Y. 1983. Morphine-induced activation of A1-dopamine neurons in the rat. *Brain Res.* **227**: 119-127.

5. MATTHEWS, R.T. & GERMAN, D.C. 1984. Electrophysiological evidence for excitation of rat ventral tegmental area dopamine neurons by morphine. *Neuroscience* **11**: 617-625.

6. BOZARTH, M.A. & WISE, R.A. 1981. Heroin reward is dependent on a dopaminergic substrate. *Life Sci.* **29**: 1881-1886.

7. WISE, R.A., SPINDLER, J., DE WIT, H. & GERBER, G.J. 1978. Neuroleptic-induced "anhedonia" in rats: pimozide blocks the reward quality of food. *Science* **201**: 262-264.

8. WISE, R.A., SPINDLER, J. & LEGAULT, L. 1978. Major attenuation of food reward with performance-sparing doses of pimozide in the rat. *Can. J. Psychol.* **32**: 77-85.

9. MEREU, G., FADDA, F. & GESSA, G.L. 1984. Ethanol stimulates the firing rate of nigral dopaminergic neurons in unanesthetized rats. *Brain Res.* **292**: 63-69.

10. GESSA, G.L., MUNTONI, F., COLLU, M., VARGIU, L. & MEREU, G. 1985. Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. *Brain Res.* **348**: 201-203.

11. DI CHIARA, G. & IMPERATO, A. 1985. Ethanol preferentially stimulates dopamine release in the *nucleus accumbens* of freely moving rats. *Eur. J. Pharmacol.* **115**: 131-132.

12. IMPERATO, A. & DI CHIARA, G. 1986. Preferential stimulation of dopamine release in the *nucleus accumbens* of freely moving rats by ethanol. *J. Pharmacol. Exp. Ther.* **239**: 219-228.

13. LUMENG, L., HAWKINS, T.D. & LI, T.K. 1985. New strains of rats with alcohol preference and non preference. In: *Alcohol and aldehyde metabolizing system*. R.G. Thurman, J.R. Williamson, H.R. Drott & B. Chance (Eds). Academic Press, New York. pp. 537-544.

14. LI, T.K., LUMENG, L., Mc BRIDE, W.J. & MURPHY, J.M. 1987. Rodent lines selected for factors affecting alcohol consumption. In: *Advances in biomedical alcohol research*. K.O. Lindros, R. Ylikahri & K. Kiianmaa (Eds). Pergamon Press, Oxford. pp. 91-96.

15. FADDA, F., ARGIOLAS, A., MELIS, M.R., SERRA, G. & GESSA, G.L. 1980. Differential effects of acute and chronic ethanol on dopamine metabolism in frontal cortex, caudate nucleus and *substantia nigra*. *Life Sci*, **27**. 979-986.

16. ROSSETTI, Z.L., MERCURO, G. & RIVANO, C.A. 1983. A study of the factor affecting flow gradient analysis of catecholamines DOPA and DOPAC by ion-pair liquid chromatography with electrochemical detection. *Life Sci.* **33**: 2387-2395.

17. FADDA, F. & GESSA, G.L. 1985. Role of dopamine in the CNS effect of ethanol. In: *Progress in alcohol research*. S. Parvez *et al.* (Eds). VNU Science Press, Utrecht. pp. 147-161.

18. CARLSSON, A., MAGNUSSON, T., SVENSSON, T.H. & WALDECK, B. 1973. Effect of ethanol on the metabolism of brain catecholamines. *Psychopharmacology* **30**: 27-36.

19. KAROUM, F., WYATT, R.J. & MAJCHROWICZ, E. 1976. Brain concentration of biogenic amine metabolites in acutely treated and ethanol-dependent rats. *Br. J. Pharmacol.* **56**: 403-411.

20. WISE, R.A. 1981. Brain dopamine and reward. In: *Theory in psychopharmacology*. S.J. Cooper (Ed.). Academic Press, New York. pp. 103-122.