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COMMUNICATIONS

Effect of acute ethanol treatment on transmitter synthesis and metabolism in central dopaminergic neurons

GONZALO BUSTOS, ROBERT H. ROTH*†, *Laboratory of Pharmacology, Department of Neurobiology, Catholic University Santiago, Chile and *Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, Connecticut, 06510, U.S.A.*

Recent studies in small animals indicate that some of the central actions of ethanol might be partly mediated by an effect on brain catecholamines (Carlsson, Engel & Svensson, 1972). Furthermore, it has been suggested that these effects of ethanol may occur due to a stimulating action of ethanol on the synthesis of catecholamines in the brain secondary to an increased impulse flow in catecholamine-containing neurons (Carlsson & Lindqvist, 1973). This view is supported by the fact that ethanol given to rats enhances the synthesis of brain catecholamine, particularly dopamine, measured as the accumulation of L-3,4-dihydroxyphenylalanine (dopa) after inhibition of aromatic amino acid decarboxylase (Carlsson & Lindqvist, 1973). However, it is now well appreciated that an increase in impulse flow as well as an inhibition of impulse flow in dopamine neurons result in a marked increase in dopamine synthesis (Walters, Roth & Aghajanian, 1973; Roth, Walters & Aghajanian, 1973; Roth, Walters & Morgenroth, 1974; Roth, Walters & others, 1975). Therefore, ethanol might increase dopamine synthesis also as a consequence of inhibition of impulse flow in dopamine-containing neurons. The short-term accumulation of dihydroxyphenylacetic acid (dopac), a dopamine metabolite, in the neostriatum seems to provide a useful index of changes in functional activity of dopaminergic neurons in the nigroneostriatal pathway (Roth & others, 1973; 1974; Roth, Murrin & Walters, 1975). An increase in impulse flow in these neurons leads to an increase in the accumulation of dopac, and a decrease in impulse flow produces a reduction in dopac accumulation. In an attempt to define more clearly the interaction between ethanol and dopaminergic neurons we have studied the effect of acute ethanol treatment on dopac accumulation in the neostriatum. Waldeck (1974) has reported that ethanol administered to mice produced no effect on catecholamine synthesis as measured by the accumulation of dopa following inhibition of the decarboxylase. The discrepancy between the later results and those of Carlsson & Lindqvist in rats (1973), have led us to study also the effect of acute ethanol treatment upon dopamine synthesis in rat neostriatum.

† Correspondence.

Male Charles River Sprague-Dawley rats (200–250 g) were used. Ethanol, 20% (w/v), was administered intraperitoneally. Control animals were injected with saline (0·9% w/v). The amino acid decarboxylase inhibitor Ro-4-4602 (seryltrihydroxy-benzylhydrazine, Hoffman-La Roche, Inc., Nutley, N.J., U.S.A.) was administered intraperitoneally (800 mg kg^{-1}). At various times after treatment, the rats were decapitated and the striata rapidly dissected over ice as described by Bunney, Walters & others (1973) and kept frozen at −70°. Dopa was isolated and measured fluorometrically by the technique of Kehr, Carlsson & Lindqvist (1972) modified as described by Walters & Roth (1974). Neostriatal tissues from one rat brain was used for each dopa determination. Dopac determinations were made using a modification of the method of Murphy, Robinson & Sharman (1969) as described by Walters & Roth (1972). Striatal tissue from two rats was pooled for each dopac assay. Data were analysed with a criterion for significance of $P<0.05$ using a two-tailed *t*-test.

To determine the effect of ethanol treatment on dopamine synthesis, a technique developed by Carlsson, Kehr & others (1972) was used, in which the accumulation of dopa following inhibition of dopa decarboxylase is followed to obtain an approximation of the rate of dopamine synthesis. The technique also provides an *in vivo* estimate of tyrosine hydroxylase activity. With this technique, it has been shown that the accumulation of dopa in the neostriatum is linear for up to 60 min after injection of Ro-4-4602 (800 mg kg^{-1}) (Walters & Roth, 1974). To show the effect of ethanol pretreatment on the accumulation of dopa in the neostriatum following dopa decarboxylase inhibition, two doses of ethanol (2 and 4 g kg^{-1}, i.p.) were administered and the decarboxylase inhibitor Ro-4-4602 was given 15 min later. After another 30 min the rats were killed and the dopa measured. The results showed that there was no significant increase in the accumulation of dopa compared to saline-treated rats.

Table 1 shows the accumulation of striatal dopac, after 30 min injection of various doses of ethanol. Ethanol, 4 g kg^{-1} increased significantly the accumulation of dopac in the neostriatum compared to saline controls. A positive correlation coefficient ($r = 0.95$)

Table 1. *Effect of ethanol treatment on the accumulation of dopac in the rat neostriatum.* Ethanol was administered intraperitonally 30 min before death. The striata were dissected out and dopac was isolated and analysed fluorometrically. Control rats were injected with saline solution instead of ethanol. All results are expressed as the mean ±s.e.m. n equals the number of experiments.

Treatment	n	Dopac $\mu g\ g^{-1}$
Saline	6	1·09 ± 0·06
Ethanol (1 $g\ kg^{-1}$)	4	1·21 ± 0·06*
Ethanol (2 $g\ kg^{-1}$)	6	1·23 ± 0·08*
Ethanol (4 $g\ kg^{-1}$)	4	1·80 ± 0·11**

* Not significantly different from saline-treated rats.
** $P<0·001$ when compared to saline-treated rats.

between the dopac concentrations and the dose of ethanol was also found.

It has been shown previously that stimulation, either by electrical or pharmacological means, of the dopamine neurons originating in the zona compacta of the substantia nigra and projecting to the neostriatum results in a rapid accumulation of dopac in the neostriatum (Roth, & others, 1973; 1974; 1975). Conversely, a decrease in dopac accumulation is produced by inhibiting impulse flow in the nigroneostriatal pathway either pharmacologically or by electrolytic lesion. It seems likely then that the increase in dopac accumulation after ethanol reported here may be mediated via an increase in impulse flow in dopaminergic neurons although other mechanisms are plausible. The fact that free dopac is not transported out of the brain by an active probenecid-sensitive transport mechanism (Guldberg & Broch, 1971; Roffler-Tarlov, Sharman & Tegerdine, 1971; Wilk, Watson & Travis, 1975a; Wilk, Watson & Glick, 1975b) suggests that the increase in dopac observed is not a result of an effect of ethanol on transport.

Our finding that ethanol treatment had no effect upon dopamine synthesis as measured by the dopa accumulation technique seems at first surprising since augmented impulse flow in dopaminergic neurons usually results in an increase in dopamine synthesis as well as accumulation of dopac in the neostriatum (Murrin & Roth, 1973). It might be that ethanol is blocking the impulse flow mediated increase in dopamine synthesis. We have recently reported that ethanol added *in vitro* to striatal slices blocked the K^+-depolarization induced formation of newly synthesized dopamine while having no effect upon newly synthesized dopamine in non-depolarized slices (Gysling, Bustos & others, 1976). Furthermore, ethanol seems to exert this latter effect by inhibiting the K^+-depolarization induced activation of striatal tyrosine hydroxylase (Bustos, Roth & Morgenroth, submitted for publication). An allosteric activation of striatal tyrosine hydroxylase also results as a consequence of electrical stimulation of the nigroneostriatal pathway in the rat (Murrin, Morgenroth & Roth, 1974). In view of these observations it seems possible that ethanol may be interacting with dopaminergic neurons at least in two ways: (a) by increasing impulse flow and, (b) by inhibiting the allosteric activation of tyrosine hydroxylase and therefore the increase in dopamine synthesis mediated by impulse flow.

Carlsson & Lindqvist (1973) have reported that ethanol administered to rats enhances the rate of tyrosine hydroxylation in dopaminergic neurons. In mice, however, ethanol produced no effect on the accumulation of dopa following inhibition of the decarboxylase (Waldeck, 1974). This discrepancy was attributed by Carlsson, Engel & others (1974) to species differences. Our experiments are not in agreement with this suggestion since the studies reported here have also been performed in rats. The control rats in the experimental design used by Carlsson & Lindqvist (1973) received only the decarboxylase inhibitor and therefore they were injected once instead of twice as in our study. Due to the importance of stress in these types of studies, the possibility should be considered that the dopa accumulated in their control rats may have been lower than the true values. In any event the present communication adds support to the hypothesis of Carlsson & Lindqvist (1973) that some of the central actions of ethanol could be mediated as a result of increased impulse flow in central catecholamine-containing neurons.

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Effect of adrenergic neuron blocking agents and biguanides on the efflux of extragranular noradrenaline from adrenergic nerves in rabbit atria

D. M. PATON, *Department of Pharmacology, University of Alberta, Edmonton, Alberta TG6 2H7, Canada*

The efflux of (—)-[^{3}H]noradrenaline from reserpine- and pargyline-pretreated rabbit atria was accelerated by phenethylamine and tryptamine derivatives, possibly as a result of accelerative exchange diffusion (Paton, 1973a, 1975; Paton & Pasternak, 1974). The most potent phenethylamines studied were β-phenethylamine, (+)- and (—)-amphetamine and phentermine (Paton, 1975). All the tryptamine derivatives studied were much less potent (Paton, 1973a). The potency of phenethylamines and tryptamines was reduced by hydroxylation or *o*-methylation on the ring, by β-hydroxylation and by *N*-substitution. In the present study, we have examined the effects of adrenergic neuron blocking agents and related biguanides on the efflux of extragranular (—)-[^{3}H]noradrenaline in order to further elucidate the structural requirements for acceleration of efflux.

As described previously (Paton, 1973b), atria, from reserpine pretreated rabbits, were exposed to pargyline (5×10^{-4}M for 30 min) and tropolone (10^{-4}M throughout), and thereafter to 10^{-7}M or 5×10^{-7}M (—)-[^{3}H] noradrenaline for 60 min (in the continued presence of tropolone). Tissues were then blotted and transferred every 5 min to fresh media at 37°. Drugs were added between 60–90 min of efflux because, during this period, efflux occurs predominantly from adrenergic nerves (Paton, 1973b). Tropolone was present throughout the efflux. At 90 min tissues were removed, blotted and the [^{3}H]noradrenaline remaining in the tissues (in pmol g^{-1} wet weight) was then determined.

It can be seen (Table 1) that, at 10^{-4}M, all the adrenergic neuron blocking agents studied increased the efflux of (—)-[^{3}H]noradrenaline significantly, the most potent agents being bretylium and guanethidine. However, all were less potent than *p*-tyramine. The biguanidines, metformin (*NN*-dimethyldiguanide) and phenformin (phenethyldiguanidine), were inactive at 10^{-4}M. The adrenergic neuron blocking agents had significantly less effect on efflux at less than 10^{-4}M.

The effects on efflux of β-phenethylamine, (±)-phenylethanolamine and (±)-β-hydroxyphenethylguanidine were also examined (Table 2). This study illustrated the tendency for potency to be reduced by β-hydroxylation as noted previously (Paton, 1975), and the marked additional reduction in potency resulting from the guanidine substitution on the terminal nitro-