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EFFECT OF ETHANOL ON DOPAMINE SYNTHESIS AND RELEASE FROM RAT CORPUS STRIATUM*

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Abstract—The effect of ethanol upon dopamine (DA) synthesis and release from dopaminergic terminals was studied. Slices from rat corpus striatum were incubated in freshly oxygenated Krebs–Ringer phosphate (KRP) media of variable ionic composition containing L-tyrosine-^{14}C (U) as dopamine precursor and in the presence and absence of ethanol (0·1 to 0·8%, w/v). The addition of ethanol directly to normal KRP media produced no effect on the conversion of ^{14}C-tyrosine to ^{14}C-DA. As reported previously, the absence of Ca^{2+} from the incubation media markedly increased the formation of ^{14}C-DA. The presence of ethanol in this media was not able either to block or to potentiate the Ca^{2+}-free induced formation of ^{14}C-DA. The presence of K^+ (55 mM) in the incubation media also increased about 2-fold the formation of ^{14}C-DA. Ethanol (0·2 to 0·8%, w/v) added directly to the KRP-high K^+ markedly blocked the K^+-induced formation of newly synthesized ^{14}C-DA. The presence of ethanol did not modify the amount of ^{14}C-tyrosine taken up by striatal slices incubated either in normal KRP or KRP-high K^+ media. A superfusion system was used to study both spontaneous and K^+-induced release of labeled DA from striatal slices. The addition of ethanol (0·4 to 0·8%, w/v) to the superfusion system was not able either to block or to potentiate the K^+-induced release of ^{3}H-DA previously taken up by the slices nor the K^+-induced release of newly synthesized ^{3}H-DA. The results reported in this work, as well as others recently reported by Murrin *et al.* (*Pharmacologist* **16**, 128, 1974), suggest the existence of another regulatory mechanism of DA synthesis besides the commonly accepted one of feedback inhibition exerted by DA upon the rate-limiting enzyme, tyrosine hydroxylase. The possibility is also raised that the inhibitory effect of ethanol described in this paper might play a role in the intoxicating effect of ethanol *in vivo*.

Recent indirect evidence has suggested that the central actions of ethanol may be partly mediated by an effect on brain noradrenergic and dopaminergic neurons. Thus, Blum *et al.* [1] have measured ethanol-induced sleeping time in mice after the administration of chemical agents known to alter the levels of brain monoamines. Pretreatment of mice with the catecholamine synthesis inhibitor α-methyl-*p*-tyrosine (α-MPT) significantly increased the "sleeping" time induced by ethanol. This effect diminished considerably when α-MPT was administered in combination with L-Dopa, a precursor of brain noradrenaline (NA) and dopamine (DA). On the other hand, the role played by brain monoamines in the stimulating action of low doses of ethanol has been investigated by Carlsson *et al.* [2]. Low doses of ethanol stimulate motor activity in mice and rats. This effect is prevented when these animals are pretreated with α-MPT. In search of a possible neurochemical explanation for these results, we thought it would be of interest to look at the action of ethanol upon aspects that control the life cycle of brain monoamines. The dopaminergic nigro-striatal system was chosen for these studies.

Our work [3, 4] and that of others [5–7] indicate that it is possible to study DA synthesis and release in resting and K^+-depolarized slices from rat striatum. The purpose of this paper, therefore, is 2-fold: (1) to study the effect of ethanol on DA synthesis in striatal slices incubated in media of different ionic composition; and (2) to study the effect of ethanol upon K^+-induced release of labeled DA from rat corpus striatum.

MATERIAL AND METHODS

Studies on DA synthesis. Sprague–Dawley rats from both sexes, weighing about 200–250 g, were decapitated, and the left and right striata were rapidly dissected. Striatal tissue slices (0·18 mm in thickness) were prepared with a Sorvall tissue chopper. Tissue slices, weighing about 30–40 mg, were incubated at 37° in beakers containing 5 ml of either Krebs–Ringer phosphate (KRP), pH 7·4, or KRP-high K^+ (55 mM), pH 7·4, or Ca^{2+}-free KRP, pH 7·4, saturated with 95% O_2 + 5% CO_2. After a 10-min preincubation period, labeled L-tyrosine-^{14}C (U) with a sp. act. of 5 mCi/m-mole was added to the media, producing a final tyrosine concentration of $2{\cdot}5 \times 10^{-5}$ M. Thereafter, the slices were incubated an additional 30 min, the beakers were chilled on ice, and slices were separated from the media by centrifugation at 10,000 rev/min in a Sorvall refrigerated centrifuge. The slices were then homogenized in 15% trichloroacetic acid (TCA); each incubation media was acidified with 0·5 ml of 50% TCA.

Carrier DA, NA and tyrosine (50 μg of each) were added to both tissue homogenates and media and the precipitated protein was then removed by centrifugation at 10,000 rev/min for 20 min. The tissue and

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B.P. 25/2—D

media extracts were immediately frozen and kept for separation and analysis for labeled DA and its metabolites as described below. Under the experimental conditions used, the apparent K_m for ^{14}C-tyrosine was $2{\cdot}4 \times 10^{-6}$ M and the synthesis of ^{14}C-DA was linear for up to 45 min when using a ^{14}C-tyrosine concentration of $2{\cdot}5 \times 10^{-5}$ M. Tissue blanks were run by incubating striatal slices as described above but in the presence of α-MPT (2×10^{-4}M), an inhibitor of tyrosine hydroxylase. Results were calculated as described previously and are expressed in terms of nmoles ^{14}C-DA/g wet weight/hr.

Similar results were obtained when tissue blanks were run by incubating striatal slices which had been initially precipitated with 15% TCA.

Studies on DA release. Ten mg of striatal slices was incubated for 30 min at 37° in 2 ml KRP, pH 7·4, saturated with 95% O_2 + 5% CO_2 and containing either ^{3}H-tyrosine (2×10^{-7} M) or ^{3}H-DA (3×10^{-7} M). The slices were then transferred to superfusion chambers (2-ml capacity) from which the release of exogenously taken up ^{3}H-DA or newly synthesized ^{3}H-DA was followed essentially as described before [4]. The slices were washed with 5 ml KRP and then superfused with KRP solution which was being continuously oxygenated and prewarmed to 37°. A constant flow of 4 ml/min was maintained by means of a roller pump. Stimulation of release was carried out for 1 min by switching the superfusion to isosmotic KRP containing K^+ (55 mM).

Potassium-induced release from striatal slices of newly formed ^{3}H-DA was started after 10 min of superfusion with normal KRP. When the release from striatal slices of exogenously loaded ^{3}H-DA was to be studied, an initial superfusion period of 12 min was allowed before release was stimulated with K^+. These procedures allowed for a constant and steady basal release prior to stimulation. Samples containing the released material were collected every min into tubes containing 1 ml of 50% TCA and carrier DA and NA (50 μg of each). Catechols were extracted from the tissues with 15% TCA. Samples were immediately frozen and kept for chromatographic analysis. Release of radiolabeled DA was expressed as the percentage of total ^{3}H-DA found in the tissue and in different collecting tubes at the end of the superfusion period.

Chromatographic procedures and radioactivity determinations. Separation and analysis of the tissue and the media for labeled DA and its metabolites were carried out by adsorption chromatography through Alumina columns and ion-exchange chromatography through Amberlite CG-120 as described previously [4]. Alumina columns were used to concentrate the catecholamines and deaminated metabolites and to separate them from tyrosine and *O*-methylated metabolites. Long Amberlite CG-120 columns (14 × 0·4 cm) were used to separate DA from deaminated metabolites and from L-Dopa. Eluates from the columns containing labeled DA were analyzed for ^{14}C and ^{3}H in a Nuclear Chicago Scintillation counter to a constant deviation of 1·5% (analysis through Amberlite columns showed that labeled DA accounted for 80–85 per cent of the labeled catechols synthesized or released after K^+ stimulation). Reported values have not been corrected for recovery.

The ^{14}C-tyrosine taken up by the tissue during the incubation period was also determined [3]. Tyrosine was separated from catechols and other labeled tyrosine metabolites by passage through Alumina columns and columns of Dowex 50 W − × 8 (H^+), 100–200 mesh. Eluates from the columns containing labeled tyrosine were analyzed for ^{14}C as described above.

Solutions and chemicals. The Krebs–Ringer phosphate used in these experiments had the following composition: NaCl, 128 mM; KCl, 4·8 mM; $CaCl_2$, 0·75 mM; $MgSO_4$, 1·20 mM; glucose, 16 mM; Na_2HPO_4, 16 mM at pH 7·4; sodium ascorbate, 20 mg/liter. Krebs–Ringer phosphate-high K^+ was made by replacing proportions of NaCl with equimolar amounts of KCl. Other modifications of the KRP are described in the text.

L-Tyrosine-^{14}C (U), L-tyrosine-3,5-^{3}H and ^{3}H-dopamine were obtained from New England Nuclear Corp.

RESULTS

Effect of ethanol on dopamine synthesis. Table 1 shows that ethanol (0·8%, w/v) added directly to normal KRP produced no significant effect on the total conversion of ^{14}C-tyrosine to ^{14}C-DA by striatal slices. As reported previously [6], the absence of

Table 1. Effect of ethanol on synthesis of dopamine by striatal slices*

Incubation media	n	^{14}C-dopamine synthesis (nmoles/g wet wt/hr) Tissue	Media	Total	Tissue/Media
KRP	14	32·4 ± 1·2	3·3 ± 1·0	35·6 ± 2·5	9·8
KRP + ethanol (0·8%, w/v)	8	30·8 ± 1·6	4·5 ± 0·4	35·4 ± 2·3	6·8
KRP, Ca^{2+}-free	10	43·2 ± 2·6†	1·9 ± 0·9	45·2 ± 2·3†	22·7
KRP, Ca^{2+}-free + ethanol (0·8%, w/v)	6	43·0 ± 3·7†	2·0 ± 0·7	45·0 ± 2·5†	21·5
KRP-high K^+ (55 mM)	9	42·9 ± 1·6†	11·8 ± 0·6†	54·7 ± 2·0†	3·62
KRP-high K^+ (55 mM) + ethanol (0·8%, w/v)	10	31·7 ± 2·5‡	11·6 ± 0·8†	43·4 ± 2·9†,‡	2·5

* Striatal slices were prepared by means of a Sorvall tissue chopper and incubated in media containing saturating concentrations of ^{14}C-tyrosine ($2{\cdot}5 \times 10^{-5}$ M, sp. act. 5·0 mCi/m-mole and 1·25 μCi/flask) for 30 min at 37°. ^{14}C-dopamine was separated by column chromatography, and its radioactivity determined in a Nuclear Chicago scintillation counter. Results represent the mean ± S. E. M.; n represents the number of different experiments.

† $P < 0{\cdot}001$ when compared to respective normal KRP control.

‡ $P < 0{\cdot}001$ when compared to KRP-high K^+ (55 mM) without ethanol.

Table 2. Effect of ethanol on ^{14}C-tyrosine uptake by striatal slices*

Incubation media	^{14}C-tyrosine uptake (nmoles/g wet wt/hr)
KRP	418·0 ± 48·9
KRP + ethanol (0·8%, w/v)	500·5 ± 42·9†
KRP-high K^+ (55 mM)	421·6 ± 34·0‡
KRP-high K^+ (55 mM) + ethanol (0·8%, w/v)	392·2 ± 34·0†

* The striatum was dissected out and slices were prepared by means of a Sorvall tissue chopper. Incubation conditions were as described under Table 1. ^{14}C-tyrosine taken up by the tissues was separated by column chromatography, and its radioactivity determined in a Nuclear Chicago scintillation counter. Results represent the mean ± S. E. M. of seven different experiments.

† Not significantly different when compared to respective control without ethanol.

‡ Not significantly different when compared to respective normal KRP control.

Ca^{2+} ions from the KRP significantly increased the formation of newly synthesized ^{14}C-DA by striatal slices (Table 1). The absence of Ca^{2+} from the incubation media also markedly raised the tissue/media ratio of ^{14}C-DA, probably due to the inhibitory effect of lack of Ca^{2+} on the release of newly formed ^{14}C-DA [4]. The presence of ethanol in this media was not able either to block or to potentiate the Ca^{2+}-free-induced formation of ^{14}C-DA.

Depolarizing concentrations of K^+ ions (55 mM) increased about 2-fold the formation of ^{14}C-DA by striatal slices and significantly increased the amount of newly synthesized ^{14}C-DA present in the media (Table 1). Potassium ions (55 mM) also markedly lowered the tissue/media ratio of ^{14}C-DA. This last effect is due both to the stimulatory effect of high K^+ on the release [4] of newly synthesized DA and to its inhibitory effect on the re-uptake of the monoamine [8]. Ethanol (0·8%, w/v) added directly to the KRP-high K^+ media markedly blocked the K^+-induced formation of newly synthesized ^{14}C-DA (Table 1; column 5). This effect seems to be even more striking when only the ^{14}C-DA present in the tissue is analyzed (Table 1; column 3). The presence of ethanol in the KRP-high K^+ media also seemed to lower the tissue/media ratio of ^{14}C-DA (Table 1; column 6).

It is possible that an inhibitory effect of ethanol on K^+-induced formation of newly synthesized ^{14}C-DA could have been produced through an inhibitory effect of ethanol on ^{14}C-tyrosine uptake by striatal slices. In order to test this, we measured the ^{14}C-tyrosine taken up by the tissue at the end of the incubation period. The results are shown in Table 2 and indicate that the presence of ethanol (0·8%, w/v) in the media does not modify the amount of ^{14}C-tyrosine taken up by striatal slices incubated either in normal KRP or KRP-high K^+ (55 mM) media.

As shown in Table 3, ethanol exerts its inhibitory effect on K^+-induced formation of newly formed ^{14}C-DA even at concentrations as low as 0·2% (w/v). At this concentration ethanol produces a 33 per cent inhibition on the K^+-induced synthesis of ^{14}C-DA.

Table 3. Effect of different doses of ethanol on K^+-induced formation of newly synthesized ^{14}C-DA by striatal slices*

Incubation media	n	Total ^{14}C-dopamine synthesis (nmoles/g wet wt/hr)	Inhibition of high K^+ effect on dopamine synthesis† (%)
Normal KRP	14	35·6 ± 2·5	
KRP-high K^+ (55 mM)	9	54·7 ± 2·0‡	0
KRP-high K^+ (55 mM) + ethanol (0·1%, w/v)	6	55·6 ± 1·8	0
KRP-high K^+ (55 mM) + ethanol (0·2%, w/v)	6	48·4 ± 1·4§	33
KRP-high K^+ (55 mM) + ethanol (0·4%, w/v)	6	44·3 ± 1·8‖	52
KRP-high K^+ (55 mM) + ethanol (0·8%, w/v)	10	43·4 ± 2·9‖	59

* The striatum was dissected out and slices were prepared by means of a Sorvall tissue chopper. Incubation conditions and separations of ^{14}C-dopamine were as described under Table 1. Results represent the mean ± S. E. M.; n represents the number of different experiments.

† High K^+ effect on dopamine synthesis is equal to ^{14}C-DA synthesized in a KRP-high K^+ media minus ^{14}C-DA synthesized in normal KRP control.

‡ $P < 0·001$ when compared to respective normal KRP control.

§ $P < 0·005$ when compared to KRP-high K^+ (55 mM) without ethanol.

‖ $P < 0·001$ when compared to KRP-high K^+ (55 mM) without ethanol.

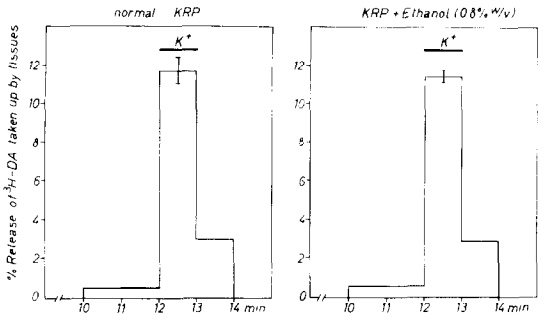

Fig. 1. Effect of ethanol on K^+-induced release of exogenous ^{3}H-DA from striatal slices. Striatal slices were incubated in normal KRP for 30 min at 37° in the presence of ^{3}H-DA and then transferred to a superfusion system. Release of ^{3}H-DA was then measured in either normal KRP (left side of the figure) or KRP + ethanol (right side of the figure). Potassium stimulation is shown by the dark lines at the top of the figure, and it was carried out for 1·0 min after following spontaneous release for 12 min. The tissues had taken up an average of 284,048 ± 19,263 and 312,854 ± 13,300 cpm of ^{3}H-DA (mean ± S. E. M.). The figure represents the mean ± S. E. M. of three different experiments.

High concentrations of ethanol (0·4 and 0·8%, w/v) produce a 60 per cent inhibition (Table 3). Very low concentrations of ethanol (0·1%, w/v) are not able to inhibit the K^+-induced formation of ^{14}C-DA (Table 3).

Effect of ethanol on dopamine release. Other events produced in the dopaminergic nerve terminal by K^+ depolarization were also studied. Figure 1 and Table 4 show the effect of ethanol on K^+-induced release of exogenously taken up ^{3}H-DA and newly formed ^{3}H-DA. In these experiments, the striatal slices were incubated in the presence of the tracer as described under Methods, and the K^+-induced release was followed both in the presence and absence of ethanol. Under these experimental conditions, K^+ stimulation produced a marked increase in the release of ^{3}H-DA (Fig. 1 and Table 4). The addition of ethanol (0·8%, w/v) to the superfusion media was not able either to block or to potentiate the K^+-induced release of labeled DA from tissues previously loaded with ^{3}H-DA (Fig. 1) and the K^+-evoked release of newly synthesized ^{3}H-DA (Table 4). Ethanol at a concentration of 0·4% (w/v) also produced no effect on K^+-evoked release of ^{3}H-DA.

DISCUSSION

The results described in this paper confirm recent reports indicating that it is possible to use striatal slices as a simple experimental tool to study DA synthesis and release in dopaminergic terminals of the rat striatum [3–7]. We have used this system to analyze the action of ethanol upon dopaminergic neurons in the brain.

Recent reports have shown that an increase in impulse flow in dopaminergic neurons, as in other central monoaminergic neurons, results in an increase in the synthesis, catabolism and turnover of DA [9, 10]. A cessation of impulse flow, on the other hand, leads to a reduction in the release and catabolism of DA and to a paradoxical increase in DA synthesis [11, 12]. It seems possible to reproduce partially these phenomena *in vitro* using brain slice techniques. Thus, incubation of striatal slices in a K^+-enriched medium (55 mM) enhanced the release of newly synthesized DA and accelerated the synthesis of DA (Table 1). The acceleration in the DA synthesis rate was accounted for by the fact that neuronal depolarization mediated by K^+ enhances the release of newly formed DA, which, in turn, relieves the rate-limiting enzyme, tyrosine hydroxylase, from end-product inhibition [5]. The removal of Ca^{2+} ions from the KRP also causes a marked increase in DA synthesis in striatal slices (Table 1). This last effect has been shown to be specific for dopaminergic terminals, since the absence of Ca^{2+} ions has no effect on the synthesis of norepinephrine in cortical slices [6, 13]. Roth *et al.* [12] have presented evidence which indicates that the absence of Ca^{2+} from the incubation media causes an allosteric activation of striatal tyrosine hydroxylase. Therefore, these investigators have suggested that the increase in DA synthesis observed after cessation of impulse flow in dopaminergic terminals is due to a diminished influx of Ca^{2+} ions into the terminals.

Table 4. Effect of ethanol on K^+-induced release of newly synthesized ^{3}H-dopamine from striatal slices*

Superfusion media	Release of ^{3}H-DA (per cent of newly synthesized ^{3}H-DA) Release 1 min before K^+ stimulation	Release during K^+ stimulation
KRP normal	0·36 ± 0·05	3·72 ± 0·12
KRP + ethanol (0·8%, w/v)	0·46 ± 0·06†	3·39 ± 0·10†

* Striatal slices were incubated in normal KRP for 30 min at 37° in the presence of ^{3}H-tyrosine, and then transferred to the superfusion system. Spontaneous and K^+-induced release from the tissues was then followed in the superfusion system in either the absence or presence of ethanol. Potassium stimulation was carried out for 1·0 min. The tissues exposed to KRP or KRP + ethanol had previously synthesized, respectively, 241,592 ± 19,161 and 200,636 ± 21,212 cpm of ^{3}H-DA (mean ± S. E. M.). Values in the table represent the mean ± S. E. M. of three different experiments.

† Not significantly different when compared to respective control without ethanol.

Ethanol (0·8%, w/v) produced no significant effect on the formation of ^{14}C-DA by striatal slices incubated in a normal KRP medium (Table 1). Ethanol was also found to be without effect on the increased formation of ^{14}C-DA induced by the absence of Ca^{2+} ions from the incubation media. However, ethanol added directly to a K^+-enriched medium (55 mM) markedly inhibited the K^+-induced formation of newly synthesized ^{14}C-DA by striatal slices (Tables 1 and 3). It is possible that an inhibitory effect of ethanol on K^+-induced formation of ^{14}C-DA could have resulted if ethanol significantly inhibited the uptake of ^{14}C-tyrosine into the striatal slices. We feel, however, that this is not the case, since ethanol does not alter the amount of ^{14}C-tyrosine found in the tissue at the end of the incubation either in normal KRP or KRP-high K^+ medium (Table 2). It could be argued that we are not measuring the specific activity of labeled tyrosine precursor in the slices and that the tyrosine utilized for DA synthesis probably represents only a small fraction of the total tissue tyrosine pool. However, the incubation of cortical slices in KRP-high K^+ (53 mM) medium was shown not to alter the specific activity of tissue tyrosine compared to slices incubated in normal medium [5]. Moreover, the fact that ethanol does not alter the synthesis of ^{14}C-DA measured in normal medium or CA^{2+}-free medium argues against the possibility that the inhibitory effect of ethanol on K^+-induced formation of ^{14}C-DA might have been due to a decrease in the tyrosine uptake into the slices.

It is also possible that ethanol is producing its inhibitory effect on K^+-induced formation of ^{14}C-DA by acting through any of the nerve membrane changes associated with K^+ depolarization such as changes in the concentration of or membrane permeability to Na^+ ions and changes in the influx of Ca^{2+} ions. However, there is no sound experimental evidence to support this: (1) only very high concentrations of ethanol (2 to 4%, w/v), higher than the ones used in this work (0·2 to 0·8%, w/v), show any significant effect upon changes in nerve membrane that lead to an increased passive permeability for Na^+ and K^+ ions during the action potential, [14–16]; (2) the K^+ effect on catecholamine synthesis was shown, in cortical and striatal slices, not to be dependent upon the reduced Na^+ concentration in the K^+-enriched medium or on permeability of the tissue to Na^+, since neither restoring the Na^+ concentration to normal nor prior treatment with tetrodoxin antagonized the K^+ stimulation of catecholamine biosynthesis [5]; and (3) ethanol (0·4 to 0·8%, w/v) was not able either to block or to potentiate the K^+-evoked release of labeled DA from striatal slices (Fig. 1 and Table 4), a process which has been shown to be highly dependent on the presence of Ca^{2+} in the medium and completely blocked by Ca^{2+} removal [4].

Extracellular K^+ concentrations of 55 mM, such as the one used in this work to induce depolarization, most likely produce an activation of the transport enzyme responsible for the active transport of Na^+ and K^+ across the cell membrane, the $(Na^+ + K^+)$-dependent ATPase [17]. This activation of the $(Na^+ + K^+)$-ATPase seems to be responsible, in turn, for the augmented oxygen consumption in brain slices that is observed after K^+ depolarization [18–20]. The possibility exists, then, that ethanol is inhibiting the K^+-induced synthesis of DA by exerting first an inhibitory effect upon the activated $(Na^+ + K^+)$-ATPase. In fact, studies by Järnefelt [21] and Israel *et al.* [22] have shown that 0·4 to 1% ethanol inhibits the $(Na^+ + K^+)$-ATPase from rat brain. However, the inhibitory effect of ethanol on $(Na^+ + K^+)$-ATPase was diminished by increasing the concentration of K^+ in the medium. When K^+ was held constant at 40 mM, there was no observable effect of ethanol on $(Na^+ + K^+)$-ATPase at any Na^+ concentration between 5 and 240 mM [22]. On the other hand, the inhibitory effect of ethanol on K^+-induced synthesis of ^{14}C-DA is observed in the presence of 55 mM K^+ (Tables 1 and 3). These findings suggest that the inhibitory effect of ethanol reported in this work is independent of an action upon the $(Na^+ + K^+)$-ATPase. However, the effect of ethanol and its competition with respect to K^+ should be studied using the $(Na^+ + K^+)$-ATPase from rat striatum as the source enzyme in order to substantiate this point further.

Another possible mechanism to explain the ethanol effect is that the inhibition of K^+-induced DA synthesis might be due, in turn, to an inhibition by ethanol of the K^+-evoked release of endogenous or newly synthesized DA, which normally acts intraneuronally to partially inhibit tyrosine hydroxylase [5]. As shown in Fig. 1 and Table 4, K^+ (55 mM) efficiently releases exogenously labeled DA or newly synthesized DA from striatal slices. Previous reports have shown that the release of labeled DA is Ca^{2+}-dependent and that removal of the Ca^{2+} or increasing the Mg^{2+} concentration antagonizes the K^+-induced release of DA [4]. Ethanol was found not to alter this process, which suggests that the inhibition of K^+ stimulation of DA biosynthesis is not mediated via an alteration of DA release. Theoretically, an increase in DA re-uptake by nerve terminals after ethanol treatment might also account in part for the inhibition in K^+-induced DA synthesis observed after ethanol. However as shown in Table 1, the tissue/media ratio of ^{14}C-DA seems to be lower for the ethanol-treated slices as compared to controls. Moreover, ethanol seems to inhibit rather than to stimulate neurotransmitter uptake into brain slices, although rather high concentrations are needed to produce this effect [23, 24]. How then is the ethanol effect upon K^+-induced ^{14}C-DA synthesis explained? Depolarization of dopaminergic nerve terminals results in an acceleration of dopamine synthesis due to an increase in the activity of the rate-limiting enzyme tyrosine hydroxylase [5]. Until very recently this increase in tyrosine hydroxylase activity was believed to arise as a result of the removal of end-product inhibition subsequent to the release of a small pool of endogenous or newly formed dopamine which normally acts to partially inhibit tyrosine hydroxylase [5–7]. The results described in this paper are not consistent with this hypothesis, since ethanol was found to block the K^+-induced synthesis of DA while having no effect on K^+-induced release of DA. In fact, recent experiments by Murrin *et al.* [25] show that an increase in impulse flow in the nigro-neostriatal pathway of the rat causes an increase in DA synthesis, which is mediated in part by an allosteric activation of striatal

tyrosine hydroxylase. This allosteric activation appears to be mediated by an increase in affinity of the enzyme for both substrate and pteridine cofactor and a decreased affinity of the enzyme for the end-product inhibitor, dopamine. Potassium depolarization of striatal slices also results in kinetic alterations in tyrosine hydroxylase similar to those observed upon electrical stimulation of the nigro-neostriatal pathway.* Moreover, ethanol (0·2 to 0·8%, w/v) was able to block specifically the kinetic activation of tyrosine hydroxylase after K^+ depolarization, while having no effect on tyrosine hydroxylase activity from non-depolarized striatal slices.* At the present time, this seems the most likely explanation for the inhibitory effect of ethanol on K^+-induced DA synthesis. It seems quite possible that alterations in endogenous levels of cAMP which occur during depolarization of dopaminergic terminals might be responsible, in part, for the increase in DA synthesis [26, 27] and for the kinetic activation of tyrosine hydroxylase which occurs during augmented impulse flow in dopaminergic neurons [28]. Whether ethanol is inhibiting K^+-induced DA synthesis by acting first through cAMP production and/or action is currently under investigation in our laboratory. It remains to be established, also, whether ethanol blocks the activating effect on dopamine synthesis produced by other depolarizing agents or if it is specific to high K^+ concentrations.

As shown in Table 3, ethanol produced an inhibitory effect upon K^+-induced ^{14}C-DA synthesis even at concentrations as low as 0·2% (w/v) or 40 mM. These ethanol concentrations are compatible with a mild intoxication *in vivo* [29, 30]. It might be then that the intoxicant effect of ethanol is somehow related to its inhibitory action on K^+-induced neurotransmitter biosynthesis. However, before reaching any final decision regarding this point, it should be borne in mind that most likely acetaldehyde and hormonal alterations also play a role in the neuropharmacological effects produced by ethanol *in vivo* [31, 32]. Work is now in progress in our laboratory to answer these questions.

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REFERENCES

1. K. Blum, J. H. Merritt, J. E. Wallace, R. Owen, J. W. Hahn and I. Galler, *Curr. ther. Res.* **14**, 324 (1972).
2. A. Carlsson, J. Engel and T. H. Svenson, *Psychopharmacologia* **26**, 307 (1972).
3. G. Bustos, M. J. Kuhar and R. H. Roth, *Biochem. Pharmac.* **21**, 2649 (1972).
4. G. Bustos and R. H. Roth, *Br. J. Pharmac. Chemother.* **46**, 101 (1972).
5. J. E. Harris and R. H. Roth, *Molec. Pharmac.* **7**, 593 (1971).
6. M. Goldstein, T. Backstrom, Y. Ohi and R. Frankel, *Life Sci.* **9**, 919 (1970).
7. F. Javoy, Y. Agid, D. Bouvet and J. Glowinski, *J. Pharmac. exp. Ther.* **182**, 454 (1972).
8. J. E. Harris and R. J. Baldessarini, *Life Sci.* **13**, 303 (1973).
9. N. E. Anden, H. Corrodi, K. Fuxe and U. Ungerstedt, *Eur. J. Pharmac.* **15**, 193 (1971).
10. L. C. Murrin and R. H. Roth, *Pharmacologist* **15**, 514 (1973).
11. R. H. Roth, J. R. Walters and G. K. Aghajanian, *Frontiers in Catecholamine Research*, p. 567. Pergamon Press, Oxford (1973).
12. R. H. Roth, J. R. Walters and V. H. Morgenroth, III, *Neuropsychopharmacology of Monoamines and Their Regulatory Enzymes*, (Ed. E. Usdin), p. 369. Raven Press, New York (1974).
13. J. E. Harris and R. H. Roth, *Fedn Proc.* **29**, 941 (1970).
14. C. M. Armstrong and L. Binstock, *J. gen. Physiol.* **48**, 265 (1964).
15. J. W. Moore, W. Ulbricht and M. Takata, *J. gen. Physiol.* **48**, 279 (1964).
16. P. Rosenberg and T. R. Podleski, *Biochim. biophys. Acta* **75**, 104 (1963).
17. R. Whittam, *Biochem. J.* **84**, 110 (1962).
18. C. A. Ashford and K. C. Dixon, *Biochem. J.* **29**, 157 (1935).
19. R. Whittam and D. M. Blond, *Biochem. J.* **92**, 147 (1964).
20. D. M. Blond and R. Whittam, *Biochem. J.* **97**, 523 (1965).
21. J. Järnefelt, *Annls Med. exp. Biol. Fenn.* **39**, 267 (1961).
22. Y. Israel, H. Kalant and I. Laufer, *Biochem. Pharmac.* **14**, 1803 (1965).
23. M. K. Roach, D. L. Davis, W. Pennington and E. Nordyke, *Life Sci.* **12**, 433 (1973).
24. Y. Israel, F. J. Carmichael and J. A. Macdonald, *Ann. N.Y. Acad. Sci.* **215**, 38 (1973).
25. L. C. Murrin, V. H. Morgenroth, III and R. H. Roth, *Pharmacologist* **16**, 128 (1974).
26. S. Kakiuchi, T. Rall and H. McIlwain, *J. Neurochem.* **16**, 485 (1969).
27. M. Goldstein, B. Anagnoste and C. Shirron, *J. Pharm. Pharmac.* **25**, 351 (1973).
28. J. Harris, V. Morgenroth, III, R. H. Roth and R. Baldessarini, *Nature, Lond.* **252**, 156 (1974).
29. H. E. Himwich, *J. Am. med. Ass.* **163**, 545 (1957).
30. J. M. Ritchie, *The Pharmacological Basis of Therapeutics* (Eds. L. S. Goodman and A. Gilman), p. 142. McMillan, New York (1970).
31. F. W. Ellis, *J. Pharmac. exp. Ther.* **153**, 121 (1966).
32. J. Merry and V. Marks, *Lancet* **1**, 921 (1969).

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