Short communications

Effect of γ-hydroxybutyrate on the release of monoamines from the rat striatum

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A simple *in vitro* system was developed to study the effect of γ-hydroxybutyrate on nerve cell depolarization-induced release of labelled DA and 5-hydroxytryptamine. The release of ^{3}H-dopamine formed in rat striatal slices incubated with ^{3}3H-tyrosine was followed. A three minute exposure to K^+ (53·0 mM) caused a thirty-fold increase in the release of newly synthesized ^{3}H-dopamine. This K^+-induced release was antagonized when γ-hydroxybutyrate (1 mM) was present in the medium. Potassium (53·0 mM) increased (eighteen-fold) the release of ^{3}H-dopamine from striatal slices initially loaded by pre-incubation with ^{3}H-dopamine. The K^+-induced release of this pool of DA was, however, not antagonized by γ-hydroxybutyrate.

Potassium (53·0 mM) also increased the release from striatal slices of ^{3}H-5-hydroxytryptamine (5-HT) newly synthesized from ^{3}H-tryptophan. This K^+-induced release of 5-HT was also not inhibited by γ-hydroxybutyrate. The ability of γ-hydroxybutyrate to antagonize only the K^+-induced release of newly formed DA may explain why this agent causes a rapid and selective increase in brain dopamine.

The naturally occurring central nervous system depressant γ-hydroxybutyrate (GHB) (Roth & Giarman, 1970) and its lactone precursor, γ-butyrolactone (GBL), cause a marked increase in brain dopamine (DA) (Gessa, Vargiu, Crabai, Boero, Carboni & Camba, 1966; Roth & Surh, 1970). This effect appears to be rather specific in that the levels of other brain monoamines, such as noradrenaline or 5-hydroxytryptamine, and γ-aminobutyric acid are not markedly altered by anaesthetic doses of GHB (Giarman & Schmidt, 1963; Gessa *et al.*, 1966; Roth & Surh, 1970). The increase in brain DA induced by GHB parallels the regional distribution of normal endogenous DA (Gessa *et al.*, 1966). Fluorescence microscopy indicates that GHB produces this selective increase exclusively within the terminals or varicosities of the DA-containing neurons (Aghajanian & Roth, 1971). Recent *in vivo* observations suggest that GHB may increase brain DA by enhancing its binding or preventing its release from dopaminergic neurons as well as by accelerating synthesis (Roth & Suhr, 1970; Roth, 1971). Others have reported that this increase is mainly due to a stimulatory effect by GHB on DA synthesis (Spano, Tagliamonte, Tagliamonte & Gessa, 1971). As a more direct approach to this problem we wish to report a simple *in vitro* system which allowed us to study the effect of GHB on release of labelled DA and serotonin induced by nerve cell depolarization.

Methods.—Striatal tissue slices (0·18 mm in thickness) were prepared with a Sorvall tissue chopper, from the striatum of adult, male Sprague-Dawley rats. Tissue slices weighing about 70 mg, were incubated for 30 min at 37° C in 5·0 ml of Krebs-Ringer-Phosphate (KRP), pH 7·4, saturated with 95% O_2+5% CO_2 and containing either L-tyrosine-3,5-^{3}H (25 ci/mM; 0·04 μM), 3,4-dihydroxyphenylethyl-1-^{3}H (*N*)-Amine (8·3 ci/mM; 0·1 μM) or L-tryptophan-^{3}H (5·3 ci/mM; 0·4 μM). At the end of the incubation period, the slices were transferred to a lucite chamber with a nylon mesh bottom (pore size=35 microns) which allowed a complete and rapid separation between slices and medium, while maintaining the slices inside the chamber. After a washing period of two minutes with KRP, the release of radioactivity from the slices was followed by transferring the chambers containing the tissues through successive beakers, containing 5·0 ml of freshly oxygenated KRP. In tissues labelled with ^{3}H-tyrosine and ^{3}H-tryptophan, the spontaneous release of total radioactivity and of ^{3}H-DA and ^{3}H-5-hydroxytryptamine was followed for two consecutive periods of three minutes each, before nerve cell depolarization was induced by K^+ (53·0 mM). The spontaneous release of exogenous ^{3}H-DA previously taken up into the tissues was followed for 7 consecutive periods of five minutes prior to stimulation with K^+ (53·0 mM). Separation and analysis of the

media for labelled DA and its metabolites, was carried out by absorption chromatography through alumina columns and ion exchange chromatography through Amberlite CG-120 columns as described previously (Roth & Stone, 1968). Analysis of labelled 5-hydroxytryptamine release in the media was carried out by ion exchange chromatography through Dowex 50×4 (Na^+) (Costa, Spano, Grappetti, Algeri & Neff, 1968). Eluates from the columns, containing labelled DA and 5-hydroxytryptamine, were analysed for tritium in a Packard liquid scintillation spectrometer.

Results.—The addition of K^+ (53·0 mM) to the medium for a period of three minutes resulted in a thirty-fold or greater increase in the efflux of newly synthesized DA from striatal slices previously incubated with ^{3}H-tyrosine (Fig. 1). In four experiments K^+ produced a mean increase in release of (3,210±590 cpm/min)/100 mg of striatum. Upon return to a medium with normal K^+ the release of labelled DA gradually came back to basal levels. Analysis by ion-exchange chromatography revealed that more than 80% of the label released during exposure to high K^+, was accounted for by ^{3}H-DA. The addition of γ-hydroxybutyrate (1 mM) substantially diminished the release of newly synthesized ^{3}H-DA induced by K^+ (Fig. 1). Under the experimental conditions used, this inhibitory effect was of the order of 27±6·1% ($P<0.01$ for an average of four experiments). GHB was found to have no effect on the spontaneous release of newly synthesized ^{3}H-DA. GHB at a concentration of 0·1 mM had no significant effect on the K^+-induced release of newly synthesized DA. Other experiments also showed that GBL (1 mM), the lactone precursor of GHB, had no effect on spontaneous or K^+ induced release of newly formed ^{3}H-DA.

Potassium (53·0 mM) also produced an eighteen-fold increase in the release of exogenously loaded ^{3}H-DA. Spontaneous release before stimulation was of the order of 1,200 (cpm/min)/100 mg striatum, and it was not affected by the presence of GHB. GHB (1 mM) produced no effect on the K^+-induced release of exogenously loaded ^{3}H-DA (a non-significant increase of 8·8±10·4% for an average of four experiments was observed).

The spontaneous and K^+-induced release of ^{3}H-5-hydroxytryptamine newly synthesized from ^{3}H-tryptophan was also studied in the presence of GHB. Potassium stimulation produced a 75% increase in the release of ^{3}H-5-hydroxytryptamine (from 400±32 to 700±97 (cpm/min)/100 mg striatum) and GHB was found to have no significant effect on this release or on the spontaneous release of ^{3}H-5-hydroxytryptamine (average of three experiments).

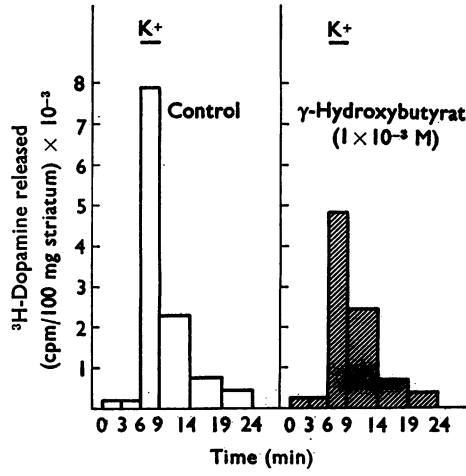

FIG. 1. Typical pattern of spontaneous and K^+-induced release of ^{3}H-DA newly synthesized from ^{3}H-tyrosine in absence and presence of GHB. Striatal slices were incubated in KRP for 30 min, at 37° C in the presence of ^{3}H-tyrosine, as described in the text, and then transferred to lucite chambers in which release of ^{3}H-DA was followed for a total period of 24 minutes. Stimulation by K^+ (53·0 mM) is shown by the dark line in top of figure.

Discussion.—The results presented in this communication indicate that under the experimental conditions used, GHB has a blocking effect on the K^+-induced release of newly formed DA while having no effect on the release of exogenously loaded DA or newly formed 5-hydroxytryptamine. It is noteworthy that the concentration of GHB necessary to inhibit dopamine release *in vitro* is similar to the concentration of GHB found in the brain after administration of exogenous GHB at a time when the rats lose the righting reflex (Giarman & Roth, 1964) and when the DA levels in the subcortex begin to increase (Walters & Roth, 1971).

The ability of GHB to antagonize selectively the release of newly synthesized DA is of interest for several reasons. First, to our knowledge this is the only drug which appears to antagonize selectively the release of central dopamine. Secondly, this effect of GHB may explain in part why this agent causes a rapid and selective increase in the brain level of DA and an apparent increase in the rate of DA biosynthesis (Spano *et al.*, 1971 ; Walters & Roth, 1971). Thirdly, studies with this drug appear to indicate that the release of DA synthesized from tyrosine differs from the release of exogenous DA previously taken up into striatal slices. This posssibility is further strengthened by experiments in our laboratory which indicate that the potassium-induced release of newly synthesized DA is markedly antagonized by the removal of Ca^{++} from the media while the potassium-induced release of exogenously loaded DA is less sensitive to removal of Ca^{++} (Bustos & Roth, unpublished observations). Fourthly, from a clinical standpoint, in view of the recent suggestion that the symptoms associated with Huntington's Chorea might, in part, result from a hypersensitivity of DA receptors in the striatum to DA (Klawans, 1970), drugs such as GHB which appear to block selectively the release of DA might be of some beneficial use in the treatment of this disorder.

The lactone form of the drug, GBL, when added to the incubation media had no effect on the release of newly synthesized DA from striatal slices. This *in vitro* finding appears to extend previous observations from this and other laboratories indicating that GBL must be converted to GHB by a blood lactonase before it is able to exert an effect on brain dopamine and on the behaviour of the animal (Roth, Delgado & Giarman, 1966 ; Gessa, Vargiu, Crabai, Bezzi & Camba, 1967 ; Guidotti & Ballotti, 1970).

Finally, the fact that GHB blocked the release of newly synthesized DA but not newly formed 5-hydroxytryptamine is in agreement with the *in vivo* finding in which no striking increase in the brain level of 5-hydroxytryptamine was found after GHB (Gessa *et al.*, 1966 ; Roth & Surh, 1970). The reasons for this specificity of action as well as the detailed mechanism by which GHB blocks the release of newly formed DA remains to be clarified.

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