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DOPAMINERGIC NEURONS: EFFECTS ELICITED BY γ-HYDROXYBUTYRATE ARE REVERSED BY PICROTOXIN

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In 1966, Gessa and coworkers made the interesting observation that γ-hydroxybutyrate (GHB) caused a dose dependent increase in the levels of brain dopamine (1). Some years later it was realized that the increase in endogenous striatal dopamine resulted due to the fact that GHB caused a reversible suppression of impulse flow in the nigro-neostriatal dopamine neurons (2,3). Dopamine neurons paradoxically increase synthesis in response to a cessation of impulse flow (3-7). This increase in dopamine synthesis coupled with an attenuated release explains the rapid increase in the steady state levels of dopamine found in the dopaminergic nerve terminals when impulse flow is blocked in these neurons. The observed increase in transmitter synthesis occurs as a result of an increase in tyrosine hydroxylase activity (3,5,7). This activation of tyrosine hydroxylase produced by a cessation of impulse flow appears to be mediated by changes in the affinity of the enzyme for substrate, pterin cofactor and dopamine (8,9) and can be reversed *in vitro* by addition of calcium. However, the actual mechanism by which GHB blocks impulse flow in the nigro-neostriatal dopamine neurons is uncertain, although some studies suggest that the drug is acting directly on neurons within the substantia nigra. For example, direct injection of GHB into the striatum has little or no effect on tyrosine hydroxylase activity or dopamine levels while injection into the substantia nigra produces a dramatic increase in striatal dopamine levels and tyrosine hydroxylase activity (3,10). The injection of a local anesthetic such as xylocaine or injection of γ-aminobutyric acid (GABA) into the substantia nigra also caused a rapid and marked increase in striatal dopamine. Since iontophoretic application of GABA onto dopamine cells in the zona compacta has been shown to inhibit spontaneous activity of these cells (11) the above observations were not unexpected because both drug treatments in theory should effectively block impulse flow in the nigro-neostriatal neurons. These observations have, however, led to the speculation that GHB may block impulse flow in central dopaminergic neurons by acting as a GABA agonist at central synapses (10).

In view of the close structural similarity between GHB and GABA this seems like a likely possibility, although at present no direct evidence exists to support this contention. Therefore, in order to test this hypothesis more directly we examined the effects of picrotoxin, a known GABA antagonist (12), on the ability of GHB to increase tyrosine hydroxylase activity in the neostriatum and olfactory tubercles, two brain areas rich in dopaminergic nerve terminals (13). The following is an account of such a study and supports in part the contention that GHB may exert its effects on dopaminergic neurons by mimicking the action of GABA at central synapses.

Male Sprague Dawley rats obtained from Charles River, Inc., were injected with drugs and killed by decapitation. γ-Butyrolactone (GBL, Matheson, Coleman and Bell) was administered in preference to the sodium salt of GHB since it is more rapidly and uniformly absorbed following i.p. injection. Once in the blood stream GBL is rapidly converted to GHB by plasma and liver lactonase (14). The neostriatum and olfactory tubercles were dissected out as described previously and frozen on dry ice (15,16). In vivo tyrosine hydroxylase activity was determined by following the short term (30-45 min) accumulation of DOPA after inhibition of DOPA decarboxylase by administration of RO-4-4602 (seryl-trihydroxybenzylhydrazine, 800 mg/kg, i.p.). DOPA was assayed fluorometrically after isolation from tissue extracts by ion exchange column chromatography (6,17).

Administration of GBL in a dose (400 mg/kg) which causes a block of impulse flow in both the nigro-neostriatal and mesolimbic dopaminergic neurons (2,3, Walters and Roth, unpublished data) and a significant increase in endogenous dopamine, caused within 30 minutes a 90% and 40% increase in DOPA accumulation in the neostriatum and olfactory tubercles respectively (Table I).

Table I. Reversal of the Increase in Dopa Accumulation by Picrotoxin*

	DOPA Levels (ng/g)	
	Striatum	Olfactory Tubercle
Controls	1271 ± 118 (6)	1049 ± 60 (17)
GBL (400 mg/kg)	2420 ± 155 (6)	1465 ± 100 (6)
Picrotoxin (3 mg/kg)	1640 ± 62 (7)	1411 ± 89 (3)
GBL (400 mg/kg) + Picrotoxin (3 mg/kg)	1582 ± 118 (6)	970 ± 94 (3)
GBL (400 mg/kg) + Picrotoxin (6 mg/kg)	1770 ± 169 (6)	1152 ± 130 (3)

*Results are expressed as the mean ± S.E.M. Values in parentheses indicate the number of individual experiments.

The ability of GBL to increase DOPA accumulation was dramatically antagonized by pretreatment with picrotoxin. Picrotoxin in a dosage of 3 mg/kg produced a 73% blockade of the GBL induced increase in DOPA accumulation in the striatum and a complete blockade in the olfactory tubercle. It is worthy of note that picrotoxin itself causes a significant increase in DOPA accumulation. Since an increase in impulse flow is known to enhance dopamine synthesis by activation of tyrosine hydroxylase, this effect of picrotoxin is most likely due to the ability of this agent to increase impulse flow in the nigro-neostriatal and mesolimbic dopaminergic neurons by removal of the tonic inhibitory GABA-ergic influence on these dopaminergic systems. Recent electrophysiological studies have now demonstrated that picrotoxin does increase the firing rate of both nigrostriatal and mesolimbic dopamine neurons (18; Nowycky and Roth, unpublished observations).

These results are consistent with the hypothesis that GHB may cause an inhibition of dopamine cell firing by acting as a GABA agonist at central synapses within the substantia nigra. This action may be mediated by the ability of GHB to directly interact with GABA receptors on the dopamine cell bodies or dendrites within the substantia nigra resulting in an inhibition of the spontaneous activity of these cells. However, these biochemical studies are not sufficient to conclude at which central site GHB is exerting its GABA agonistic activity or if in fact GHB is indeed acting as a GABA agonist. Although unlikely, it is conceivable that the generalized seizure activity produced by picrotoxin is sufficient to cause sporadic depolarization of the dopamine nerve terminal thus reversing the effects of GHB (19). More extensive studies comparing and contrasting the ability of other GABA antagonists and non-specific convulsive agents to block the biochemical effects elicited by GHB will help to answer this question. In addition iontophoretic and single unit recording studies will hopefully clarify the central site of action as well as the mechanism by which GHB blocks impulse flow in central dopaminergic neurons.

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