Title : RO-21-3981, A BENZODIAZEPINE, AND GABA METABOLISM IN RAT BRAIN SYNAPTOSOMES

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Introduction. Pharmacological effects of diazepam are presumably mediated through the facilitation of GABA (γ-aminobutyrate) action in brain[1]. It is not clear if it acts directly on GABA-receptors[1]. RO-21-3981 (Midazolam; 8-chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo-[1,5-a][1,4]benzodiazepine maleate) has been developed as a water soluble bezodiazepine anesthetic agent. We studied the effects of this drug on GABA metabolism using the rat brain synaptosomes as a model. It was anticipated that this drug would inhibit GABA metabolism and the results would conform with our hypothesis that the resultant accumulation of GABA is instrumental in causing anesthesia[2].

Overall GABA Metabolism. Synaptosomes were prepared from rat forebrain by the sucrose density centrifugation method[3]. They were layered between 0.9-1.2M sucrose and, after collection and recentrifugation, resuspended in an incubation medium containing 10mM succinate. They (0.5mg protein) were incubated in the presence of 10μM [1-^{14}C]GABA (0.5μCi/ml) for 1 hour at 30° and the $^{14}CO_2$ produced was measured[4]. ID_{50} was approximately 0.15mM (ID_{10} = 0.025mM) with a control value of 3.56±0.10nmoles/mg protein (N = 226). Since this inhibition encompasses the processes of catabolism, release and uptake, each was assessed separately.

GABA Catabolism. GABA-transaminase activity, which is the rate-limiting step in GABA catabolism, was assayed by coupling excess aldehyde dehydrogenase and by measuring the reduction of NAD spectrophotometrically[5]. Very little inhibition of GABA-transaminase by RO-21-3981 was observed at near saturation concentrations.

GABA Release. This was studied by incubating [1-^{14}C]GABA-preloaded synaptosomes through 20 2ml washes at 2.5' intervals followed by dissolution of the synaptosomes in NaOH. The radioactivity of the washes and the radioactivity and the protein contents of the synaptosomes were determined. KCl or RO-21-3981 was added only to the 9th → 12th washes when necessary. Control release was 0.39±0.10nmoles/hr/mg protein (N = 5). KCl, which is known to cause membrane depolarization, caused an immediate release of radioactivity. RO-21-3981 caused a dose-related but delayed release of radioactivity. ED_{50} was approximately 0.5 or 0.6mM when the amounts of radioactivity released into the washes or that remained in the synaptosomes was measured. ED_{10} was in the 0.30-0.35mM range.

GABA Uptake. This was measured by incubation of synaptosomes in [2,3-^{3}H]GABA (50μM, 2μCi/ml) for 10' at 30°. At 0' and 10', aliquots were diluted in ice-cold 50mM GABA-containing Ringer's solution and centrifuged immediately at 10,000g. The difference in radioactivity in the pellets between 10' and 0' represents uptake of GABA into the synaptosomes. Inhibition by RO-21-3981 on GABA uptake was dose-related and ID_{50} was 0.10mM (ID_{10} = 0.015mM). Control uptake was 7.95± 0.67nmoles/min/mg protein (N = 10).

Summary. The summarized results at the bottom of the Table show that the inhibition of GABA uptake by RO-21-3981 is responsible for the inhibition of overall GABA metabolism (CO_2 production) by rat brain synaptosomes. The effective dose was approximately 0.1mM for a 50% effect. However, based on our correlation of MAC to ID_{10} with volatile anesthetic agents[4], it is more pertinent to examine the ID_{10} for this drug. The ID_{10} for RO-21-3981 is 15-25μM. The inhibition of GABA uptake, at this low concentration, may lead to synaptic accumulation of GABA and finally to the anesthetic state[2,4].

Table. Effects of RO-21-3981 on GABA Metabolism

mM	GABA → CO_2 % Inhibition	GABA Release % Released	GABA Release % Retained	GABA Uptake % Inhibition
0	-	15.2	75.6	-
0.01	0.4	-	-	-
0.05	18.8	-	-	31.1
0.10	46.8	-	-	48.6
0.15	51.8	-	-	60.3
0.20	67.8	-	-	68.8
0.25	91.6	18.2	72.6	-
0.30	-	-	-	73.3
0.40	-	-	-	80.7
o.50	99.6	33.5	49.0	-
0.60	-	-	-	84.9
0.75	-	76.0	27.8	-
0.80	-	-	-	90.7
1.00	-	92.6	8.0	95.4
ID_{50}	0.15mM	0.6mM	0.5mM	0.10mM
ID_{10}	0.025mM	0.35mM	0.30mM	0.015mM

*GABA-transaminase activity was not inhibited.

References.
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