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EFFECTS OF γ-AMINOBUTYRIC ACID AGONIST AND ANTAGONIST DRUGS ON LOCAL CEREBRAL GLUCOSE UTILIZATION[1]

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Abstract

The [^{14}C]2-deoxy-D-glucose method of Sokoloff et al. (Sokoloff, L., M. Reivich, C. Kennedy, M. H. Des Rosiers, C. S. Patlak, K. D. Pettigrew, O. Sakurada, and M. Shinohara (1977) J. Neurochem. *28:* 897-916) was used to study local cerebral glucose utilization (LCGU) in rats treated with γ-aminobutyric acid (GABA) agonist (muscimol and 4,5,6,7-tetrahydroisoxazolo[5,4-C]pyridin-3-ol, THIP) and antagonist (bicuculline) drugs. It was of interest to determine if the pattern of LCGU responses to GABA agonists and antagonists administered systemically *in vivo* would reflect the known distributions of markers for central GABAergic synapses. The patterns of LCGU responses to muscimol and THIP generally were similar. Most brain regions showed dose-dependent decreases in LCGU; others showed no effects; but the red nucleus showed an increase. The GABA antagonist bicuculline produced convulsions and variable LCGU responses, depending on the time of administration. Bicuculline also partially antagonized the depressant effects of muscimol on LCGU. The magnitudes and distribution of *in vivo* cerebral metabolic responses to specific GABA agonists were not correlated simply with markers for GABAergic synapses. This lack of correlation indicates that additional factors, such as neural circuitry, regulate the LCGU responses to GABAergic drugs.

γ-Aminobutyric acid (GABA), which is considered to be an important neuronal inhibitor, is the neurotransmitter for up to 40% of the neurons in the mammalian nervous system (Bloom and Iversen, 1971). Many aspects of GABA biosynthesis, storage, release, catabolism, and postsynaptic effects are widely documented (Roberts et al., 1976; Krogsgaard-Larsen et al., 1979a).

Recent techniques for immunocytochemical localization of the GABA biosynthetic enzyme, L-glutamic acid decarboxylase (Ribak et al., 1979), and for light microscopic localization of high affinity GABA receptors (Palacios et al., 1980, 1981b) have provided considerable information on the distribution of GABAergic synapses. Pre- and postsynaptic markers for GABA synapses are widely and heterogeneously distributed throughout the rat brain although their distributions are not identical. It is not known, however, how this regional variation is reflected in the central effects of GABA agonists and antagonists administered systemically *in vivo*. It is feasible to address this question because of the availability of centrally acting GABA agonists, such as muscimol and 4,5,6,7-tetrahydroisoxazolo[5,4-C]pyridin-3-ol (THIP), which cross the blood-brain barrier (Baraldi et al., 1979; Enna and Maggi, 1979). Furthermore, because local cerebral glucose utilization (LCGU) is related to cerebral functional activity (Sokoloff, 1979), the autoradiographic, [^{14}C]2-deoxy-D-glucose ([^{14}C]DG) technique of Sokoloff et al. (1977) to measure LCGU allows delineation of the brain areas that are activated or depressed by pharmacological manipulations. In this investigation, the [^{14}C]DG technique was used to study the effects on LCGU of systemic administration of GABA agonists and antagonists. A preliminary report has appeared elsewhere (Palacios et al., 1981a).

Materials and Methods

Materials. Sixty male, Osborne-Mendel rats (3 to 4 months old) were obtained from the animal facilities of

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the National Institutes of Health (Bethesda, MD). [^{14}C]DG (specific activity, 55 to 57 mCi/mmol) was obtained from New England Nuclear Corp. (Boston, MA) and was rechromatographed to ascertain purity. Muscimol and (+)-bicuculline were purchased from Sigma Chemical Co. (St. Louis, MO). THIP was a gift from Dr. S. J. Enna, University of Texas (Houston, TX). Pentobarbital (Nembutal sodium) was purchased from Abbott Laboratories (North Chicago, IL).

Preparation of rats. Under ether anesthesia, rats were prepared with indwelling femoral venous and arterial catheters as previously described (Sokoloff et al., 1977; Dow-Edwards et al., 1981). They then were placed in a sound-insulated wooden box (62 × 46 × 43 cm), where body temperature was regulated with a rectal thermoprobe connected to a feedback device (YSI Indicating Controller model 73ATA; Yellow Springs Instruments, Yellow Springs, OH) that activated heating wires on the roof of the box when body temperature fell below 36°C (London et al., 1981). The rats were allowed to recover for 4 hr before injection of the drugs or [^{14}C]DG.

Drug treatments. Prior to injection, bicuculline (3 mg) was dissolved in a few drops of concentrated HCl, and the volume was increased to 9 ml with 0.9% (w/v) NaCl. The pH was adjusted to 4 with NaOH, and the final volume was brought to 10 ml with 0.9% NaCl. All other drugs (muscimol, THIP, and pentobarbital) were dissolved or diluted in 0.9% NaCl with no pH adjustments. All drugs as well as saline (in control rats) were injected intravenously in a volume of 1 ml/kg of body weight. Treatments included: control (saline, 14 min before [^{14}C]DG), muscimol (1 to 7 mg/kg, 14 min before [^{14}C]DG), THIP (6, 12, or 24 mg, 15 min before [^{14}C]DG), bicuculline (0.3 mg/kg, 15 or 2 min before [^{14}C]DG), muscimol (4 mg/kg, 14 min before [^{14}C]DG), bicuculline (0.3 mg/kg, 15 or 2 min before [^{14}C]DG), and pentobarbital (30 mg/kg, 10 min before [^{14}C]DG).

Determination of LCGU. LCGU was determined after injection of [^{14}C]DG (125 μCi/kg of body weight, i.v.) as previously described (Sokoloff et al., 1977; Dow-Edwards et al., 1981). Rats were killed 50 min after injection of [^{14}C]DG by a pentobarbital overdose (60 mg in 1 ml, i.v.). Brains were removed and frozen in 2-methylbutane (Fisher Scientific Co., Silver Spring, MD) cooled to −60°C. Frozen, 20-μm sections were obtained by cutting the brains on a Cryo-Cut II cryostat (American Optical Co., Buffalo, NY) maintained at −20 to −22°C. Sections were picked up on glass coverslips, and radioactivity in individual brain regions was determined by quantitative autoradiography (Sokoloff et al., 1977).

LCGU was calculated from brain and plasma radioactivities and plasma glucose concentrations using rate and lumped constants for transport and phosphorylation of [^{14}C]DG as given by Sokoloff et al. (1977). Calculations were made with an operational equation that allows the arterial plasma concentration to vary during the experimental period (Savaki et al., 1980).

Statistical analysis. LCGU values obtained following the various treatments were compared by a one-way analysis of variance (Steel and Torrie, 1960). Significance of the differences between individual means was assessed by Duncan's new multiple range test (Duncan, 1957).

Results

Effects of GABA agonists. Animals injected with muscimol (1 to 7 mg/kg) or THIP (6, 12, or 24 mg/kg) appeared sedated. A dose of 10 mg/kg of muscimol produced death in less than 5 min. Occasional twitches, followed by sedation, were observed shortly after the administration of each drug.

Muscimol decreased LCGU in most brain areas (Table I; Fig. 1). LCGU fell markedly in regions of the neocortex, where maximal decreases exceeded 50% of control LCGU. Muscimol also changed the apparent LCGU pattern in the neocortex. In autoradiographs from control rats, the density of autoradiographic grains appeared the greatest in cortical layer IV, indicating that LCGU was highest in this cortical layer. This was not the case in muscimol-treated rats, where the dark band of LCGU in layer IV was no longer evident. Other regions which showed large, significant muscimol-induced decrements in LCGU (by more than 50% of control LCGU) were the striatum and medial geniculate body. A decrease of up to 50% was observed in the anterior thalamus. THIP produced similar decrements (Table II; Fig. 2). Decreases in LCGU in caudal parts of the brain, such as the superior olive, the nucleus of the lateral lemniscus, and inferior colliculus, were less pronounced than in anterior regions. Some brain regions did not show a decreased LCGU in response to GABA agonists. These included the lateral habenula and zona incerta, where there were no significant GABA agonist effects, and the red nucleus, where muscimol significantly increased LCGU.

The effects of muscimol on LCGU were dose dependent (Table I). One milligram per kg of muscimol produced variable changes, and mean LCGU did not differ significantly from control except in the red nucleus. Two and one-half milligrams per kg of muscimol produced nearly maximal effects in areas where LCGU decreased. Therefore, the ED_{50} was between 1 and 2.5 mg/kg. Only in the inferior colliculus and red nucleus did increasing the dose of muscimol above 2.5 mg/kg produce a significantly greater response. In the inferior colliculus, 7 mg/kg of muscimol reduced LCGU by an additional 17% over the reduction at 2.5 mg/kg. In the red nucleus, 1 to 4 mg/kg of muscimol increased LCGU by up to 46%, but 7 mg/kg increased LCGU by 122% (Table I). It was not possible to determine a maximal change because of the toxicity of high doses of muscimol.

THIP effects also were dose dependent (Table II). Effects similar to those obtained with doses between 1 and 2.5 mg/kg of muscimol were achieved by 12 mg/kg of THIP, whereas 24 mg/kg produced effects similar to 4 to 7 mg/kg of muscimol. This indicates an approximately 10-fold lower potency of THIP as compared to muscimol.

In order to compare the effects of direct GABA agonists with those of anesthetics, which also decrease LCGU (Sokoloff et al., 1977; Crane et al., 1978) and reportedly involve a GABAergic link in their action (Meibach et al., 1979; Nelson et al., 1980), the effects of pentobarbital on LCGU were studied. Pentobarbital (25 mg/kg, i.v.) produced a generalized decrease in LCGU; no increases were found in any area (data not shown).

Effects of bicuculline. The effects of bicuculline (0.3 mg/kg, i.v.) were studied in 13 rats. Three animals received bicuculline 15 min before [^{14}C]DG, and the other 10 received bicuculline 2 min before [^{14}C]DG. All animals convulsed within 1 min after bicuculline administration. The duration of convulsions varied among individual rats from 2 to 5 min. After the convulsions, all rats appeared sedated.

The LCGU effects of bicuculline were dependent upon the time of drug administration. Autoradiographs from animals injected with bicuculline 15 min before [^{14}C]DG looked similar to controls; however, when animals were injected 2 min before [^{14}C]DG, an increase of LCGU occurred in many brain regions. The areas showing marked increases in LCGU as compared with control (Fig. 3) were: the neocortex (where a columnar pattern

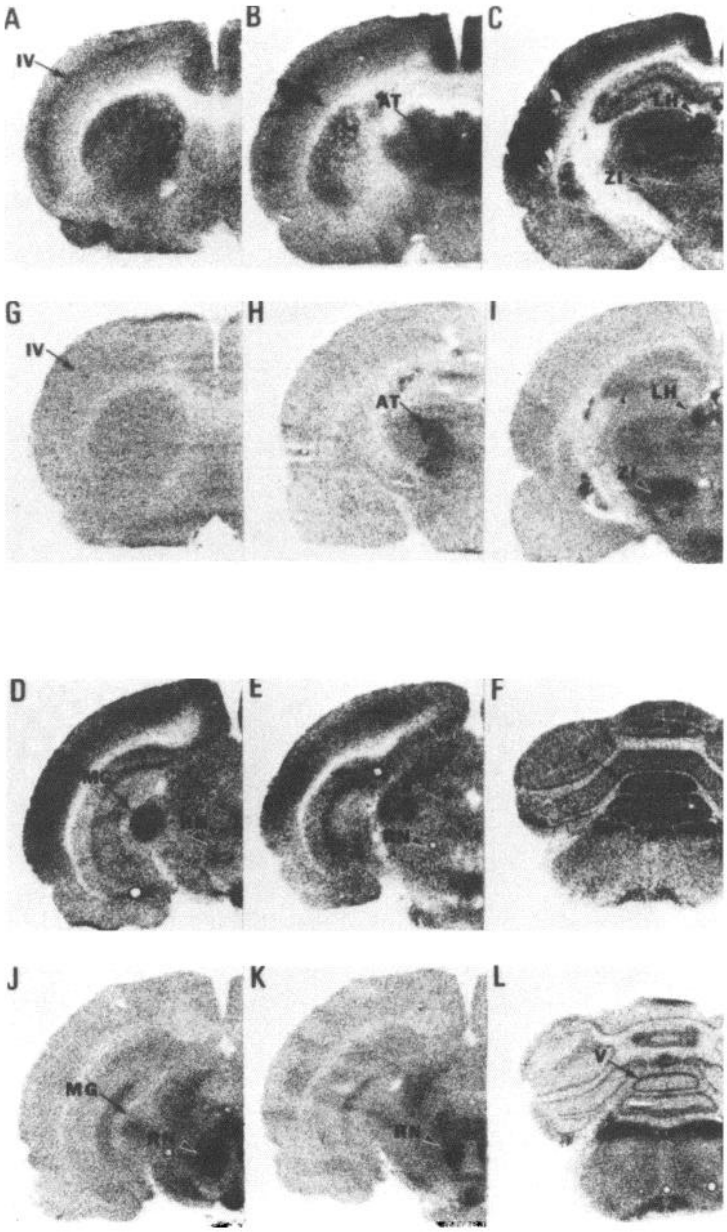

Figure 1. Effects of muscimol on autoradiographic grain densities, representing local glucose utilization, in the rat brain. Photographs of x-ray film exposed to 20-μm brain sections from rats subjected to the [^{14}C]2-deoxy-D-glucose procedure are shown. *A* to *F* show grain densities in film exposed to sections from different levels of a control brain. *G* to *L* show densities in films exposed to sections from corresponding brain levels of a rat injected with muscimol (7 mg/kg, i.v., 15 min before [^{14}C]DG). *AT*, Anterior nuclei of thalamus; *IV*, layer IV of the cerebral cortex; *LH*, lateral habenula; *MG*, medial geniculate body; *RN*, red nucleus; *V*, cerebellar vermis; *ZI*, zona incerta.

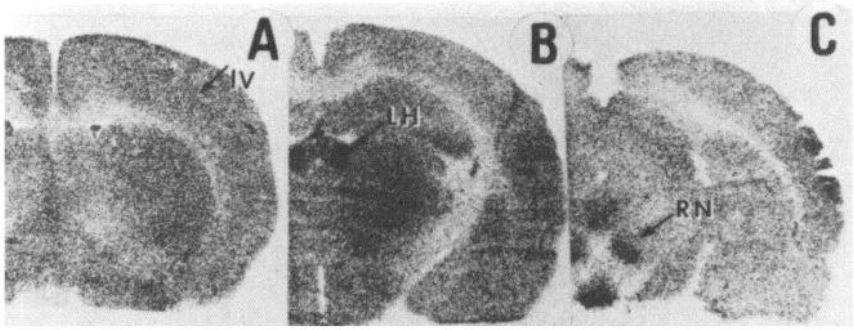

Figure 2. Effects of 4,5,6,7-tetrahydroisoxazolo[5,4-C]pyridin-3-ol on autoradiographic grain densities, representing local glucose utilization, in the rat brain. These are photographs of x-ray film exposed to 20-μm brain sections from a rat injected with THIP (24 mg/kg, i.v., 15 min before [^{14}C]2-deoxy-D-glucose) and subjected to the [^{14}C]DG procedure. *A* to *C* show grain densities in film exposed to sections from different levels of brain. Compare with *A*, *C*, and *E* in Figure 1, which represent corresponding control sections. *IV*, Layer IV of the cerebral cortex; *LH*, lateral habenula; *RN*, red nucleus.

TABLE I

Effects of muscimol on local cerebral glucose utilization

Rats were injected intravenously with muscimol (in 1 ml/kg of body weight of 0.9%, w/v, NaCl) or an equal volume of 0.9% NaCl 14 min before [^{14}C]DG. LCGU was measured as previously described (Sokoloff et al., 1977; Dow-Edwards et al., 1981). Four to 11 densitometric readings were taken from each brain region. Each number is the mean ± SEM for the number of rats indicated in parentheses.

Brain Region	Dose of Muscimol (mg/kg)				
	0	1	2.5	4	7
Cerebellum, vermis	74 ± 3.2 (7)	86 ± 13 (4)	57 ± 6.9 (5)	56 ± 8.3 (6)	56 ± 9.2 (4)
Superior olive	112 ± 7.8 (7)	136 ± 20 (4)	91 ± 13 (5)	88 ± 10 (6)	91 ± 10 (5)
Inferior colliculus	142 ± 54 (7)	157 ± 24 (4)	116 ± 14 (5)	118 ± 8.4 (6)	92 ± 7.7[a] (5)
Nucleus of lateral lemniscus	88 ± 5.4 (7)	95 ± 11 (4)	71 ± 10 (5)	76 ± 6.6 (6)	75 ± 6.2 (5)
Interpeduncular nucleus	105 ± 8.9 (7)	112 ± 17 (4)	81 ± 9.4 (5)	93 ± 12 (6)	75 ± 7.6 (5)
Medial geniculate body	104 ± 13 (7)	82 ± 14 (4)	45 ± 4.7[a] (5)	45 ± 5.3[a] (6)	44 ± 6.7[a] (5)
Red nucleus	46 ± 2.7 (7)	67 ± 11[a] (4)	47 ± 6.7 (5)	60 ± 5.3 (6)	102 ± 8.4[a] (5)
Lateral habenular nuclei	86 ± 7.9 (7)	93 ± 19 (3)	68 ± 6.4 (5)	70 ± 11 (6)	66 ± 9.8 (5)
Anterior nucleus, thalamus	70 ± 5.3 (7)	60 ± 15 (3)	36 ± 3.9[a] (5)	35 ± 4.8[a] (6)	40 ± 5.2[a] (5)
Auditory cortex	94 ± 9.9 (7)	80 ± 13 (4)	41 ± 6.8[a] (5)	44 ± 8.3[a] (5)	35 ± 5.3[a] (5)
Dentate gyrus	42 ± 7.0 (4)	40 ± 4.5 (2)	32 ± 4.8 (5)	44 ± 1.0 (2)	38 ± 7.0 (5)
Hippocampus	53 ± 2.3 (7)	58 ± 10 (3)	40 ± 6.0 (5)	45 ± 5.5 (6)	37 ± 5.3 (5)
Zona incerta	64 ± 5.8 (7)	61 ± 13 (3)	43 ± 6.8 (5)	50 ± 5.6 (6)	58 ± 1.7 (5)
Striatum	73 ± 9.1 (6)	66 ± 16 (3)	33 ± 4.1[a] (5)	41 ± 10[a] (3)	33 ± 5.8[a] (5)
Medial cortex	77 ± 11 (5)	58 ± 15 (2)	31 ± 5.4[a] (5)	44 ± 3.5 (2)	32 ± 3.2[a] (3)
Nucleus accumbens	65 ± 10 (5)	67 ± 25 (2)	33 ± 4.6 (5)	50 ± 13 (2)	38 ± 9.7 (3)
Frontal pole, cortex	71 ± 7.6 (5)	61 ± 15 (2)	32 ± 4.4[a] (5)	42 ± 2.2[a] (2)	32 ± 4.5[a] (3)
External plexiform layer, olfactory bulb	70 ± 8.2 (5)	70 ± 27 (2)	45 ± 6.6 (5)	40 ± 0 (1)	52 ± 13 (2)

[a] Significantly different from control with $p < 0.05$.

was apparent), striatum, globus pallidus, molecular layer of the dentate gyrus, substantia nigra pars reticulata, inferior colliculus (not shown), and the cerebellar flocculus (not shown). In comparison, whereas the anterior cingulate cortex showed increased LCGU, the posterior cingulate cortex had an apparently lower LCGU.

The effects of bicuculline administration on the muscimol-induced alterations in LCGU also were studied. Rats were injected with 2.5 mg/kg of muscimol 15 min before [^{14}C]DG and with 0.3 mg/kg of bicuculline 2 min before [^{14}C]DG. Although bicuculline did not reverse the muscimol effect completely, antagonism was observed (Fig. 4). This was especially notable in the medial geniculate body and auditory cortex. Conversely, preadministration of muscimol not only completely blocked bicuculline-induced convulsion but also completely abolished the increases in LCGU produced by the GABA antagonist.

Discussion

As expected from the proposed inhibitory action of GABA in the nervous system, GABA agonist administration generally decreases LCGU in the rat brain. Decreased LCGU in the rat caudate nucleus in response to GABA agonists has been noted previously (Kelly and McCulloch, 1978).

Similar LCGU patterns are produced by two different GABA agonists, and decreases in LCGU can be antago-

nized partially by bicuculline. This indicates that the effects of GABA agonists on LCGU are mediated through interactions with GABA receptors. However, agonist effects on LCGU need not be due directly to the action on GABA receptors at the site but may be secondary to GABA receptor-mediated effects at other sites which project to the site in question.

Bicuculline-induced convulsions are associated with increases in LCGU. Similar increases, with convulsions produced by other means, have been reported (Collins et al., 1976; Nelson et al., 1980; Wooten and Collins, 1980; Ben Ari et al., 1980). The fact that different LCGU effects are obtained at different times after bicuculline administration indicates that pharmacokinetics can influence the LCGU responses to drugs.

Although muscimol is degraded rapidly in the periph-

TABLE II

Effects of 4,5,6,7-tetrahydroisoxazolo[5,4-C]pyridin-3-ol on local cerebral glucose utilization

Rats were injected intravenously with THIP (in 1 ml/kg of body weight of 0.9%, w/v, NaCl) or an equal volume of 0.9% NaCl 15 min before [^{14}C]DG. LCGU was measured as previously described (Sokoloff et al., 1977; Dow-Edwards et al., 1981). Four to 11 densitometric readings were taken from each brain region. Each number is the mean ± SEM for the number of rats indicated in parentheses.

Brain Region	Dose of THIP (mg/kg)		
	0	12	24
Cerebellum, vermis	74 ± 3.2 (7)	63 ± 5.0 (4)	42 ± 4.1[a] (3)
Superior olive	112 ± 7.8 (7)	106 ± 11 (4)	88 ± 7.5 (3)
Inferior colliculus	142 ± 54 (7)	134 ± 16 (4)	99 ± 9.9 (3)
Nucleus of lateral lemniscus	88 ± 5.4 (7)	82 ± 5.0 (4)	63 ± 7.7[a] (3)
Interpeduncular nucleus	105 ± 8.9 (7)	94 ± 8.4 (4)	64 ± 5.4[a] (3)
Medial geniculate body	104 ± 13 (7)	60 ± 2.2[a] (4)	41 ± 4.8[a] (3)
Red nucleus	46 ± 2.7 (7)	55 ± 2.4 (4)	48 ± 8.8 (3)
Lateral habenular nuclei	86 ± 7.9 (7)	76 ± 4.7 (4)	67 ± 6.4 (3)
Anterior nucleus, thalamus	70 ± 5.3 (7)	48 ± 1.6 (4)	34 ± 5.2 (3)
Auditory cortex	94 ± 9.9 (7)	63 ± 4.6[a] (4)	32 ± 3.2[a] (3)
Dentate gyrus	42 ± 7.0 (4)	45 ± 3.2 (4)	27 ± 2.0 (2)
Hippocampus	53 ± 2.3 (7)	53 ± 14 (4)	32 ± 1.5[a] (3)
Zona incerta	64 ± 5.8 (7)	60 ± 3.0 (4)	41 ± 3.5 (3)
Striatum	73 ± 9.1 (6)	43 ± 4.5 (4)	32 ± 4.0[a] (3)
Medial cortex	77 ± 11 (5)	40 ± 5.0[a] (3)	28 ± 6.5[a] (2)
Nucleus accumbens	65 ± 10 (5)	43 ± 3.0 (3)	28 ± 5.5[a] (2)
Frontal pole, cortex	71 ± 7.6 (5)	42 ± 3.7 (3)	26 ± 6.0 (2)
External plexiform layer, olfactory bulb	70 ± 8.2 (5)	59 ± 6.7 (3)	42 ± 6.5 (3)

[a] Significantly different from control with $p < 0.05$.

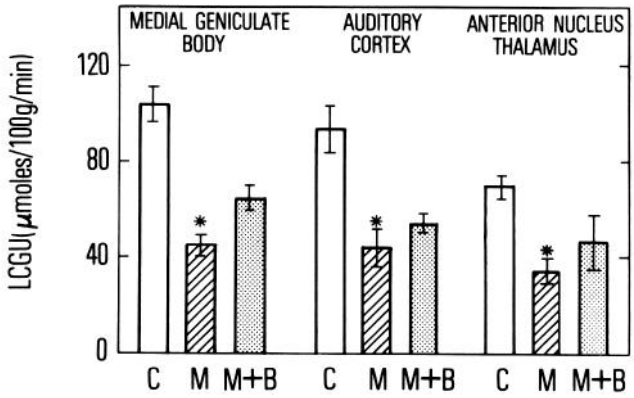

Figure 4. Effects of muscimol, alone or with bicuculline, on local cerebral glucose utilization in the medial geniculate body, auditory cortex, and anterior nucleus of the thalamus. *C*, Control rats which were injected intravenously with 1 ml/kg of body weight of 0.9%, w/v, NaCl 14 min before [^{14}C]2-deoxy-D-glucose. *M*, Rats which were injected intravenously with 2.5 mg/kg of muscimol in 1 ml/kg of 0.9% NaCl 14 min before [^{14}C]DG. *M + B*, Rats which were injected intravenously with 2.5 mg/kg of muscimol 14 min before [^{14}C]DG and with 0.3 mg/kg of bicuculline 2 min before [^{14}C]DG. LCGU was measured as previously described (Sokoloff et al., 1977; Dow-Edwards et al., 1981). Four to 11 densitometric readings were taken from each brain region. Each value represents the mean ± SEM for 7 control rats, 5 muscimol-treated rats, and 3 muscimol plus bicuculline-treated rats. *, Significantly different from control with $p < 0.05$.

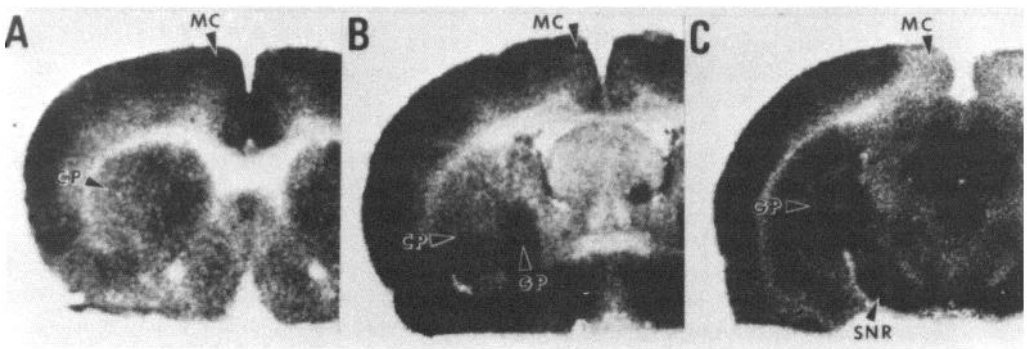

Figure 3. Effects of bicuculline on autoradiographic grain densities, representing local glucose utilization, in the rat brain. These are photographs of x-ray film exposed to 20-μm brain sections from a rat injected with bicuculline (0.3 mg/kg, i.v., 2 min before [^{14}C]DG) and subjected to the [^{14}C]DG procedure. *A*, *B*, and *C* show grain densities in film exposed to sections from different levels of brain. *CP*, Caudate-putamen, striatum; *GD*, dentate gyrus; *GP*, globus pallidus; *MC*, medial cortex; *SNR*, substantia nigra pars reticulata.

ery, a small proportion of injected muscimol seems to reach the brain (Baraldi et al., 1979; Maggi and Enna, 1979). THIP apparently is less susceptible to enzyme degradation (Krogsgaard-Larsen et al., 1979b), and its potency in inhibiting GABA binding is much lower than that of muscimol (Krogsgaard-Larsen and Arnt, 1978). This may explain the lower potency of THIP in changing LCGU. Doses of GABA agonists which alter LCGU generally agree with doses that prevent bicuculline convulsions in the rat (Matthews and McCafferty, 1979), increase neuronal firing in the pars compacta of the substantia nigra (Walters and Lakoski, 1978; Waszczak et al., 1980a, b), and increase choline uptake by the rat striatum (Ferkany and Enna, 1980). However, it is noteworthy that the ED_{50} of muscimol to inhibit the effects of 0.3 mg/kg of bicuculline is 1 mg/kg (Matthews and McCafferty, 1979). The muscimol dose tested against bicuculline in LCGU experiments was much greater (2.5 mg/kg). This probably is why bicuculline did not antagonize the muscimol effects completely in the current investigation.

One of the goals of this study was to study the possible correlations between *in vivo* responses to GABA agonists and antagonists and the anatomical distribution of markers for GABAergic synapses. It is known that pre- and postsynaptic GABAergic markers are distributed heterogeneously throughout the brain. For example, very high densities of GABAergic neurons are present in areas such as the substantia nigra, hypothalamus, and substantia innominata (Fonnum and Storm-Mathisen, 1978). High densities of GABA receptors are found in the granule layer of the cerebellum, some nuclei of the thalamus including the ventral anterior complex, the medial geniculate body, and the external layers of the cerebral cortex (Palacios et al., 1981b). However, it should be noted that autoradiographic studies of high affinity muscimol binding sites do not label other potential GABA receptors significantly. These include low affinity GABA binding sites (Enna and Snyder, 1975; Guidotti et al., 1979), bicuculline-insensitive GABA receptors (Bowery et al., 1980), and benzodiazepine-associated GABA receptors (Tallman et al., 1980; Unnerstall et al., 1981). When comparing the distribution of pre- or postsynaptic markers for GABAergic synapses with the anatomical distribution and the magnitudes of LCGU responses to GABA agonists, no simple correlation can be established. In so far as LCGU indicates functional activity, this implies that the magnitudes and *in vivo* distribution of functional responses to GABAergic drugs cannot be predicted from the distribution of GABAergic synapses. A lack of correlation between the LCGU response to systemic agonist administration and markers for a neurotransmitter system has been reported previously for the dopaminergic system. The changes after injection of dopaminergic agonists (McCulloch et al., 1979; Brown and Wolfson, 1978) are unrelated to the density of dopaminergic innervation, unrelated to the densities of dopamine receptors, and not restricted to areas receiving dopaminergic innervation. A similar lack of correlation has been noted between LCGU responses to the cholinergic, muscarinic agonist oxotremorine and the densities of muscarinic binding sites in motor system regions of the rat brain (Dow-Edwards et al., 1981). These discrepancies may reflect the fact that LCGU following systemic agonist treatment has contributions from cells receptive to the agonist as well as from projections which may or may not respond to the agonist. Because brain regions are so highly interconnected, one might have predicted the discrepancies noted above. Nevertheless, it has been noted recently that the distribution and magnitude of the LCGU response in the cerebral cortex to systemic oxotremorine apparently are related to the distributions of cholinergic, neurochemical markers, especially high affinity muscarinic receptors (Dam et al., 1982).

In a broad sense, the LCGU responses to GABA agonists resemble previously reported responses to some anesthetics. For example, thiopental and pentobarbital produce a profound, generalized decrease throughout the brain of up to 56% of control LCGU, predominantly in the gray matter (Sokoloff et al., 1977; Crane et al., 1978). γ-Butyrolactone generally reduces LCGU by up to 70% (Wolfson et al., 1977). Barbiturates modify GABAergic transmission and have been shown also to affect benzodiazepine binding (MacDonald and Barker, 1978; Skolnick et al., 1981; Leeb-Lundberg et al., 1981), indicating a possible action on some part of the GABA receptor complex. However, not all anesthetics produce a decrease in LCGU (Nelson et al., 1980).

Like barbiturates, muscimol and THIP decrease LCGU. However, unlike barbiturates, GABA agonists produce a relative activation in the red nucleus, zona incerta, and anterior thalamic nuclei. Relative increases in [^{14}C]DG uptake have been observed in these same brain regions of mice with the mutant gene tottering during focal motor seizures (Noebels and Sidman, 1979). These observations suggest that activation of these and homologous brain regions may be involved also in the observed epileptic-like EEG effects of muscimol and THIP in man and other primates (Scotti de Carolis et al., 1969; Shoulson et al., 1978; Tamminga et al., 1979; Meldrum and Horton, 1980).

The complete understanding of the effects of the GABA agonists in the brain requires elucidation of the multiple brain pathways and anatomical systems involved. Further studies involving discrete, anatomic lesions or genetic mutants may be of value. The results reported here can be useful in directing such further efforts.

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