0270-6474/84/0406-1405$02.00/0

The Journal of Neuroscience
Vol. 4, No. 6, pp. 1405–1413
June 1984

MUSCIMOL-SCOPOLAMINE INTERACTIONS IN THE RAT BRAIN: A STUDY WITH 2-DEOXY-D-[1-^{14}C]GLUCOSE[1]

PAULI HELÉN AND EDYTHE D. LONDON*,[2]

*Laboratory of Neurosciences, National Institute on Aging, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224 and *National Institute on Drug Abuse, Addiction Research Center, c/o Baltimore City Hospitals, Baltimore, Maryland 21224*

Received August 22, 1983; Revised November 29, 1983; Accepted December 6, 1983

Abstract

The 2-deoxy-D[1-^{14}C]glucose method of Sokoloff was used to measure local cerebral glucose utilization (LCGU) in rats after injections of the GABA receptor agonist, muscimol (1.6 mg/kg and 4.0 mg/kg, i.v.); the muscarinic receptor antagonist, scopolamine (0.4 mg/kg and 2.0 mg/kg, i.v.); or combinations of both drugs. The aim was to identify brain regions where functional effects of GABAergic-cholinergic interactions could be seen. As noted previously, muscimol reduced LCGU in many brain regions. In contrast, scopolamine alone had no effect on LCGU in most brain regions; however, decreases were seen in the medial geniculate body, medial thalamic nucleus, and auditory and frontal cortical areas. Scopolamine increased LCGU in the cerebellar vermis and mesencephalic reticular formation. Although muscimol alone did not significantly affect LCGU in the external plexiform layer of the olfactory bulb or the anterior, periventricular, and parafascicular thalamic nuclei, rats treated with 0.4 mg/kg of scopolamine before 4.0 mg/kg of muscimol had LCGU decrements in those brain regions. Furthermore, the muscimol-induced decrease in LCGU in the medial cortex was enhanced by prior treatment with 0.4 mg/kg of scopolamine. In contrast, in certain brain regions where muscimol alone reduced LCGU (locus ceruleus; central gray matter; striatum; ventral, medial, reuniens, and rhomboid thalamic nuclei; and the auditory cortex), scopolamine pretreatment antagonized these decrements.

These findings suggest that endogenous cholinergic and GABAergic systems act antagonistically in some brain regions. However, in other brain regions, cholinergic transmission is required for full expression of GABAmimetic effects on LCGU. This apparent positive cholinergic-GABAmimetic interaction may have bearing on identifying the cerebral loci involved in GABAmimetic antinociception.

It is now generally accepted that GABA is a major inhibitory neurotransmitter in the central nervous system (Roberts, 1980). Because GABA itself does not readily pass the blood-brain barrier (Kuriyama and Sze, 1971), GABA analogues that do enter the brain have been used to study central GABAmimetic effects. Such drugs include muscimol, SL-76002, kojic amine, and 4,5,6,7-tetrahydroisoxazolo[5,4-C]pyridin-3-ol (THIP) (Enna and Maggi, 1979; Krogsgaard-Larsen and Falch, 1981; Palacios et al., 1981b, 1982; Kelly and McCulloch, 1982; Kendall et al., 1982). The behavioral effects of GABAmimetic agents include sedation, muscle relaxation, antinociception, and an anxiolytic effect (Potashner, 1978; Bartholini, 1980; Kendall et al., 1982).

There is evidence that GABAmimetic actions involve central cholinergic systems. For example, in the rat, muscimol decreases the rate of ACh turnover in the midbrain and cortex (Zsilla et al., 1976), induces a dose-related increase in striatal ACh content (Scatton and Bartholini, 1979), and decreases striatal ACh turnover (Bartholini et al., 1981). More recently, Kendall et al. (1982) have demonstrated that the muscarinic receptor antagonist atropine reverses the antinociceptive actions of other GABAmimetic agents. This observation is consistent with the concept that the antinociceptive action

[1] We thank P. Mahone, H. Holloway, and A. Ellis for excellent technical assistance. An abstract of this work has been published (Helén, P., and E. D. London (1982) Soc. Neurosci. Abstr. *8:* 579). The Gerontology Research Center is fully accredited by The American Association for Accreditation of Laboratory Animal Care.

[2] To whom correspondence should be addressed, at The Addiction Research Center, National Institute on Drug Abuse, c/o Gerontology Research Center, Baltimore City Hospitals, Baltimore, MD 21224. E. D. L. is a collaborative investigator of The National Institute on Aging, Gerontology Research Center.

of GABamimetic agents involves the participation of muscarinic systems.

The present study was conducted to investigate the *in vivo* distribution of cerebral metabolic effects of GABAergic-cholinergic interactions in the rat. Therefore, we used the 2-deoxy-D-[1-^{14}C]glucose ([^{14}C]DG) technique of Sokoloff et al. (1977) to measure local cerebral glucose utilization (LCGU) in rats treated with the GABA agonist muscimol, the muscarinic receptor antagonist scopolamine, or both drugs. Because LCGU is related to cerebral functional activity (Sokoloff, 1979), the autoradiographic [^{14}C]DG technique allows delineation of brain areas that are activated or depressed by pharmacological manipulations. This technique has been used previously to map metabolic responses to GABA agonist and antagonist drugs in the rat brain (Kelly and McCulloch, 1982; Palacios et al., 1982).

Materials and Methods

Thirty-eight Osborne-Mendel rats (250 to 370 gm) were obtained from the animal facilities of the National Institutes of Health (Bethesda, MD). [^{14}C]DG (55 to 57 mCi/mmol) was purchased from New England Nuclear Corp. (Boston, MA) and was rechromatographed to ascertain purity. Muscimol and (−)-scopolamine hydrobromide (scopolamine) were purchased from Sigma Chemical Co. (St. Louis, MO). Pentobarbital (Nembutal sodium) was obtained from Abbott Laboratories (North Chicago, IL).

Preparation of rats. Rats were fasted for approximately 15 hr prior to surgery to stabilize plasma glucose levels. Under pentobarbital anesthesia (50 mg/kg, i.p.), catheters were surgically implanted into the left femoral artery and vein. The skin was apposed with metal clips, and the catheters exited from the left groin. The catheters were plugged until the drug treatments were administered. After surgery, each rat was restrained with a plaster cast that enabled it to move only the forequarters but prevented the rat from pulling the catheters. Rats were allowed to recover for 4 hr in a sound-insulated wooden box (62 × 46 × 43 cm), where body temperature was monitored with a rectal thermoprobe connected to a feedback device (YSI Indicating Controller model 73ATA, Yellow Springs Instrument Co., Inc., Yellow Springs, OH) that regulated body temperature by activating heating wires on the ceiling of the box when rectal temperature fell below 35°C.

Drug treatments. Drug treatments were administered about 4 hr after surgery was completed. Muscimol and scopolamine were dissolved in 0.9% (w/v) NaCl and were injected intravenously in a volume of 1 ml/kg of body weight. The same volume of saline was injected intravenously in control rats. Each rat was given two injections; the first was at 30 min before and the second was at 14 min before [^{14}C]DG. Evaluations of drug interactions were undertaken in two separate experiments that employed different doses of muscimol and scopolamine. In the first of these experiments, the muscimol dose was high (produced a maximal effect on LCGU in most brain regions; Palacios et al., 1982) and the scopolamine dose was relatively low (Stewart and Blain, 1975). The treatment groups for the first experiment were as follows: (*1*) *saline + saline:* (*2*) *saline + muscimol* (4.0 mg/kg); (*3*) *scopolamine* (0.4 mg/kg) + *saline:* (*4*) *scopolamine* (0.4 mg/kg) + *muscimol* (4.0 mg/kg). In the second experiment, we used a high dose of scopolamine (2.0 mg/kg), selected on the basis of behavioral studies (Stewart and Blain, 1975), and a lower dose of muscimol (1.6 mg/kg). Again, each rat was given two injections, 30 min and 14 min before [^{14}C]DG. The treatment groups in this second series were as follows. (1) *saline + saline:* (*2*) *saline + muscimol* (1.6 mg/kg); (*3*) *scopolamine* (2.0 mg/kg) + *saline*; and (*4*) *scopolamine* (2.0 mg/kg) + *muscimol* (1.6 mg/kg).

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). 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 (Eastman Kodak Co., Rochester, NY) at −60°C. The frozen brains were cut into 20μm sections, with a Cryo-Cut II cryostat (American Optical Co., Buffalo, NY) maintained at −20°C to −22°C. Sections were picked up on glass coverslips, and the radioactivity in individual regions was determined by quantitative autoradiography (Sokoloff et al., 1977). Anatomical structures were identified using stereotaxic atlases (König and Klippel, 1970; Pellegrino et al., 1979). Eight to 10 densitometric readings were taken from each brain region. LCGU was calculated from brain and plasma radioactivities and plasma glucose concentrations using published values for the lumped constant and rate constants for [^{14}C]DG transport and phosphorylation (Sokoloff et al., 1977), and an operational equation that considers variations in the arterial plasma concentration during the experimental period (Savaki et al., 1980).

Physiological measures. Systolic and diastolic blood pressures and pulse rate were recorded during the [^{14}C]DG experiment by connecting the arterial catheter to a strain gauge transducer (Statham Instruments Co., Hatorey, PR) which led into a paper chart recorder (Gould Recorder 2200, Gould Inc., Cleveland, OH).

Statistical analysis. LCGU values obtained after the various treatments were compared in two separate one-way analyses of variance (Steel and Torrie, 1960), corresponding to the two experiments. Statistical significance of the differences between individual means was assessed by Duncan's new multiple range test (Duncan, 1957). Values of LCGU in the control groups from the two experiments did not differ by Student's t test ($p < 0.05$). Values of pulse rate and blood pressure before and after drug treatments were compared by Student's paired t analysis. Differences were considered significant when $p \leq 0.05$.

Results

Behavioral and physiological parameters. After muscimol injection, the rats appeared to be slightly sedated, but we did not attempt to quantify the degree of sedation. Within 1 min after the low dose scopolamine injection, the rats seemed to struggle a bit within the confines of their plaster casts for a few minutes. No consistent

behavioral effects were noted after the higher dose of scopolamine.

During the [14C]DG experiment, there was a significant increase in the pulse rate from 371 ± 13 beats per minute (bpm) (mean ± SEM) to 439 ± 16 bpm within 1 to 2 min after 0.4 mg/kg of scopolamine. The higher dose of muscimol (4.0 mg/kg) significantly decreased the pulse rate from 391 ± 12 bpm to 341 ± 23 bpm; it also significantly decreased both the systolic and diastolic pressures. The systolic pressure dropped from 162 ± 8.2 mm Hg (mean ± SEM for five rats) to 141 ± 10 mm Hg, and the diastolic pressure dropped from 126 ± 7.8 mm Hg (mean ± SEM for five rats) to 106 ± 4.6 mm Hg. No significant effects on pulse rate or blood pressure values were produced by the lower dose of muscimol (1.6 mg/kg), the higher dose of scopolamine (2.0 mg/kg), or saline injections. Despite transient drug effects on cardiovascular parameters, the values of these parameters measured in drug-treated animals during the [14C]DG procedure were within the range of control values. Arterial plasma glucose concentrations during the [14C]DG procedure did not differ between treatment groups.

LCGU effects of muscimol. In general, both doses of muscimol (1.6 and 4.0 mg/kg) were equally effective in reducing LCGU (Tables I and II). Significant decreases (about 26 to 58%) occurred in the locus ceruleus, medial geniculate body, central gray matter (substantia grisea centralis), striatum, nucleus accumbens, almost all thalamic nuclei sampled, ventral hippocampal CA1, and the medial cortex. The most profound decreases were observed in the frontal and auditory cortices (48 to 69% decrease from control).

LCGU effects of scopolamine. Most regions showed no scopolamine-associated changes in LCGU (Tables I and II). However, scopolamine significantly decreased LCGU by about 18 to 37% in the medial geniculate body, medial nucleus of the thalamus, layers IV and V of the auditory cortex, and layer IV of the frontal cortex. These significant decrements were obtained with both doses of scopolamine, except in the medial geniculate body, where the 23% decrease obtained with 0.4 mg/kg of scopolamine was not significant. In contrast, the high dose of scopolamine significantly elevated LCGU over control values in the cerebellar vermis (+65%) (Fig. 1) and mesencephalic reticular formation (+44%).

LCGU effects of 0.4 mg/kg of scopolamine + 4.0 mg/kg of muscimol. In rats treated with a low dose of scopolamine and a high dose of muscimol, LCGU was reduced significantly in the external plexiform layer of the olfactory bulb (49%) (Fig. 2) and the anterior (45%), periventricular (42%), and parafascicular thalamic nuclei (32%), regions where neither of the drugs given alone produced significant LCGU effects (Table I). In the medial cortex, where muscimol alone significantly reduced LCGU (26% less than control), scopolamine + muscimol decreased LCGU further (51% less than control; 33% less than muscimol alone) (Fig. 3). In many brain regions, muscimol alone produced significant LCGU decrements that were not further affected by the combined treatment. Such areas included the striatum (47% and 50% reductions from control by muscimol alone and scopolamine + muscimol, respectively); ventral nucleus (38% and 38% respectively), ventromedial nucleus (37% and 40%, respectively), medial nucleus (41% and 50%, respectively), and posterior nucleus of the thalamus (39% and 32%, respectively); auditory cortex, layer IV (64% and 62%, respectively) and layer V (59% and 57%, respectively); frontal cortex, layer IV (52% and 56%, respectively) and layer V (48% and 51%, respectively).

LCGU effects of 2 mg/kg of scopolamine + 1.6 mg/kg of muscimol. A high dose of scopolamine prior to a low dose of muscimol did not enhance the LCGU reductions associated with muscimol alone (Table II). On the contrary, in some regions, scopolamine partially antagonized muscimol-induced decreases in LCGU. These regions included the striatum (LCGU after scopolamine + muscimol was 150% of muscimol alone, and 64% of control), medial thalamic nucleus (137% and 57%, respectively), auditory cortical layers IV (148% and 45%, respectively) and V (146% and 53%, respectively). The negative interaction between scopolamine and muscimol in the medial thalamic nucleus and auditory cortex was notable because scopolamine alone decreased LCGU in these brain regions.

Discussion

The present report describes the effects of scopolamine and muscimol alone and in combination on glucose utilization in the rat brain. The [14C]DG technique was used to identify functional interactions between muscimol and scopolamine as well as the distributions within the brain of such *in vivo* interactions.

The effects of scopolamine on LCGU in hippocampal and cortical structures (medial, auditory layer IV, frontal layers IV and V) agree well with the results of Dam et al. (1982). Weinberger et al. (1979) found a significant decrease in the auditory cortex, similarly noted in the present study in layers IV and V, whereas Dam et al. (1982) showed a nonsignificant decreased. The insignificant changes in LCGU in the superior olive, ventral thalamus, and nucleus accumbens, as well as a decrease in the medial geniculate body reported by Weinberger et al. (1979), were confirmed in this study. The decreases in LCGU after scopolamine in the inferior colliculus, frontal cortex, lateral thalamus and dentate gyrus previously noted by Weinberger et al. (1979) were not observed in the present study. These discrepancies may reflect strain differences. In the studies by Weinberger et al. (1979) and Dam et al. (1982) and in the present study, Wistar, Fischer-344, and Osborne-Mendel rats, respectively, were used. Another possible source of the discrepancies could be differences in the anatomical site of measurement (frontal cortex versus separate layers, lateral thalamus versus individual nuclei, dentate gyrus versus dorsal-ventral).

Increased body movements were observed after a low dose of scopolamine (0.4 mg/kg) but not after a high dose (2.0 mg/kg). This observation is not inconsistent with previous results, which indicate that scopolamine effects on open field behavior follow an inverted U-shaped dose-response curve (Anisman and Cygan, 1975; Stewart and Blain, 1975). However, LCGU responses to scopolamine apparently follow a different pattern. For example, the low dose of scopolamine did not affect LCGU in the

TABLE I

Effects of high dose muscimol and/or low dose scopolamine on local cerebral glucose utilization

Muscimol and/or scopolamine were injected intravenously in 1 ml/kg body weight of 0.9%, w/v, NaCl, at 14 and 30 min before [^{14}C]DG, respectively. Rats injected intravenously with equal volumes of 0.9% NaCl served as a control group. Glucose utilization was determined as previously described (Sokoloff et al., 1977; Dow-Edwards et al., 1981). Eight to 10 densitometric readings were taken from each brain region. The values for LCGU (micromoles per 100 gm per minute) are expressed as means ± SEM for the number of rats indicated in parentheses. One-way analysis of variance and Duncan's new multiple range test were applied for statistical analysis.

	Control	Muscimol (4.0 mg/kg)	Scopolamine (0.4 mg/kg)	Scopolamine (0.4 mg/kg) + Muscimol (4.0 mg/kg)
Vermis cerebelli, uvula	75 ± 2.9 (5)	81 ± 9.0 (5)	91 ± 17 (4)	68 ± 10 (6)
Nucleus gracilis	56 ± 3.6 (5)	62 ± 4.7 (5)	60 ± 6.3 (4)	56 ± 9.3 (6)
Nucleus cuneatus	76 ± 11 (5)	84 ± 8.7 (5)	85 ± 9.7 (4)	79 ± 8.5 (6)
Formatio reticularis mesencephalica	49 ± 2.2 (5)	46 ± 4.7 (5)	55 ± 7.3 (4)	49 ± 6.6 (6)
Nucleus raphe dorsalis	58 ± 4.7 (5)	66 ± 8.9 (5)	65 ± 13 (4)	48 ± 6.1 (6)
Locus ceruleus	90 ± 4.4 (5)	86 ± 9.3 (5)	102 ± 15 (4)	86 ± 12 (6)
Nucleus reticularis lateralis magnocellularis	64 ± 7.7 (5)	57 ± 4.7 (5)	57 ± 9.6 (4)	61 ± 4.2 (6)
Oliva superior	134 ± 12 (5)	131 ± 19 (5)	124 ± 22 (4)	128 ± 13 (6)
Colliculus inferior	178 ± 8.0 (5)	185 ± 20 (5)	161 ± 28 (4)	160 ± 17 (6)
Nucleus lemnisci lateralis	96 ± 5.5 (5)	108 ± 11 (5)	95 ± 16 (4)	99 ± 7.1 (6)
Nucleus interpeduncularis	110 ± 10 (5)	106 ± 2.5 (4)	112 ± 17 (4)	86 ± 8.5 (6)
Corpus geniculatum mediale	92 ± 5.2 (5)	60 ± 6.2[a] (5)	71 ± 13 (4)	57 ± 6.5[a] (6)
Nucleus ruber	62 ± 5.2 (5)	62 ± 7.0 (4)	71 ± 6.5 (4)	73 ± 13 (6)
Nucleus habenularis lateralis	113 ± 5.6 (5)	112 ± 16 (5)	123 ± 11 (4)	91 ± 8.5 (6)
Zona incerta	59 ± 4.3 (5)	40 ± 5.1 (4)	50 ± 7.9 (4)	37 ± 5.8 (6)
Substantia grisea centralis	53 ± 1.9 (5)	43 ± 3.6 (5)	50 ± 6.1 (4)	39 ± 4.4 (6)
Substantia nigra				
Pars compacta	54 ± 4.6 (5)	47 ± 7.0 (4)	59 ± 8.4 (4)	42 ± 3.3 (6)
Pars reticulata	46 ± 2.3 (5)	39 ± 4.4 (4)	57 ± 6.1 (4)	40 ± 5.3 (6)
Striatum	76 ± 4.6 (5)	40 ± 7.6[a] (5)	78 ± 9.9 (4)	38 ± 5.4[a] (6)
Nucleus accumbens	64 ± 3.1 (5)	47 ± 6.0[a] (5)	68 ± 6.8 (4)	36 ± 5.0[a] (6)
Lamina plexiformis externa bulbi olfactorii	75 ± 8.4 (5)	67 ± 10 (4)	71 ± 8.0 (4)	38 ± 8.9[a,b] (6)
Tuberculum olfactorium	55 ± 4.1 (5)	51 ± 4.9 (5)	60 ± 8.6 (4)	44 ± 7.3 (6)
Thalamus				
Nucleus anterior	62 ± 6.2 (5)	49 ± 6.0 (5)	63 ± 6.0 (4)	34 ± 3.1[a] (6)
Nucleus ventralis	78 ± 3.5 (5)	48 ± 4.6[a] (5)	87 ± 9.9 (4)	48 ± 4.3[a] (6)
Nucleus ventromedialis	81 ± 4.8 (5)	51 ± 4.9[a] (5)	86 ± 10 (4)	49 ± 5.0[a] (6)
Nucleus periventricularis	63 ± 3.9 (5)	52 ± 8.7 (5)	56 ± 5.7 (4)	36 ± 3.9[a] (6)
Nucleus medialis	82 ± 11 (5)	48 ± 8.2[a] (5)	56 ± 3.8[a] (4)	41 ± 6.0[a] (6)
Nucleus reticularis	48 ± 5.4 (5)	36 ± 4.2 (5)	64 ± 4.0[a] (4)	34 ± 3.3[a] (6)
Nucleus reuniens	61 ± 5.7 (5)	50 ± 6.9 (5)	74 ± 10 (4)	43 ± 6.1 (6)
Nucleus rhomboideus	65 ± 5.3 (5)	54 ± 7.8 (5)	78 ± 10 (4)	46 ± 5.4 (6)
Nucleus posterior	77 ± 5.2 (5)	47 ± 5.8[a] (5)	74 ± 8.1 (4)	52 ± 7.3[a] (6)
Nucleus parafascicularis	73 ± 8.6 (5)	54 ± 5.8 (5)	75 ± 9.5 (4)	50 ± 4.8[a] (6)
Hippocampus				
Gyrus dentatus dorsalis	50 ± 3.0 (5)	55 ± 8.7 (5)	46 ± 4.9 (4)	37 ± 4.0 (6)
Gyrus dentatus ventralis	59 ± 5.0 (5)	53 ± 6.9 (5)	56 ± 6.8 (4)	39 ± 6.1 (6)
CA1, dorsalis	46 ± 1.6 (5)	49 ± 8.3 (5)	45 ± 5.0 (4)	35 ± 4.5 (6)
CA3, dorsalis	52 ± 2.2 (5)	61 ± 9.9 (5)	43 ± 4.8 (4)	41 ± 5.0 (6)
CA1, ventralis	50 ± 3.2 (5)	44 ± 4.1 (5)	46 ± 5.5 (4)	33 ± 4.5 (6)
CA2 + 3, ventralis	47 ± 2.3 (5)	49 ± 3.9 (5)	44 ± 6.5 (4)	34 ± 4.2 (6)
Cortex				
Auditiva, lamina IV	124 ± 8.2 (5)	45 ± 3.3[a] (5)	85 ± 12[a] (4)	47 ± 6.3[a] (6)
Auditiva, lamina V	99 ± 5.7 (5)	41 ± 2.2[a] (5)	74 ± 9.9[a] (4)	43 ± 6.0[a] (6)
Frontalis, lamina IV	81 ± 4.9 (5)	39 ± 6.5[a] (5)	58 ± 6.7[a] (4)	36 ± 5.3[a] (6)
Frontalis, lamina V	67 ± 5.0 (5)	35 ± 6.8[a] (5)	51 ± 6.8 (4)	33 ± 4.8[a] (6)
Medialis	65 ± 3.0 (5)	48 ± 4.5[a] (5)	61 ± 6.3 (4)	32 ± 2.3[a,b] (6)

[a] Significantly different from control, $p \leq 0.05$.

[b] Significantly different from muscimol, $p \leq 0.05$.

TABLE II

Effects of low dose muscimol and/or high dose scopolamine on local cerebral glucose utilization

Muscimol and/or scopolamine were injected intravenously in 1 ml/kg body weight of 0.9%, w/v, NaCl, at 14 min and 30 min before [^{14}C]DG, respectively. Control rats were injected with equal volumes of 0.9% NaCl. Glucose utilization was measured as previously described (Sokoloff et al., 1977; Dow-Edwards et al., 1981). Eight to 10 densitometric readings were taken from each brain region. LCGU values (micromoles per 100 gm per minute) are expressed as means ± SEM for the number of rats indicated in parentheses. One-way analysis of variance and Duncan's new multiple range test were applied for statistical analysis.

	Control	Muscimol (1.6 mg/kg)	Scopolamine (2.0 mg/kg)	Scopolamine (2.0 mg/kg) + Muscimol (1.6 mg/kg)
Vermis cerebelli, uvula	78 ± 8.2 (3)	62 ± 5.7 (5)	129 ± 10[a] (5)	93 ± 9.4[b] (5)
Nucleus gracilis	54 ± 6.4 (3)	38 ± 4.0 (5)	67 ± 7.6 (5)	47 ± 4.9 (5)
Nucleus cuneatus	80 ± 2.5 (3)	59 ± 6.3 (5)	85 ± 5.2 (5)	68 ± 7.2 (5)
Formatio reticularis mesencephalica	48 ± 5.0 (3)	39 ± 3.5 (5)	69 ± 5.4[a] (5)	58 ± 6.4[b] (5)
Nucleus raphe dorsalis	71 ± 16 (3)	50 ± 4.2 (5)	81 ± 4.8 (5)	65 ± 5.7 (5)
Locus ceruleus	105 ± 4.3 (3)	70 ± 5.8[a] (5)	127 ± 11 (5)	97 ± 12 (5)
Nucleus reticularis lateralis magnocellularis	59 ± 6.7 (3)	53 ± 4.3 (5)	72 ± 4.5 (4)	60 ± 6.1 (5)
Oliva superior	143 ± 26 (3)	133 ± 17 (5)	163 ± 7.3 (5)	118 ± 7.0 (5)
Colliculus inferior	186 ± 22 (3)	146 ± 12 (5)	198 ± 10 (5)	150 ± 12 (5)
Nucleus lemnisci lateralis	105 ± 19 (3)	92 ± 12 (5)	120 ± 6.2 (5)	98 ± 6.3 (5)
Nucleus interpeduncularis	121 ± 18 (3)	101 ± 6.3 (5)	123 ± 4.8 (5)	104 ± 12 (5)
Corpus geniculatum mediale	106 ± 3.9 (3)	53 ± 5.0[a] (5)	76 ± 3.8[a] (5)	60 ± 4.7[a] (5)
Nucleus ruber	70 ± 4.6 (3)	58 ± 6.0 (5)	79 ± 4.5 (5)	73 ± 8.3 (5)
Nucleus habenularis lateralis	117 ± 5.2 (3)	101 ± 9.4 (5)	134 ± 13 (5)	127 ± 11 (5)
Zona incerta	59 ± 5.6 (3)	50 ± 5.2 (5)	73 ± 6.0 (5)	59 ± 4.1 (5)
Substantia grisea centralis	48 ± 3.5 (3)	34 ± 3.8[a] (5)	58 ± 4.0 (5)	41 ± 3.4 (5)
Substantia nigra				
Pars compacta	56 ± 4.9 (3)	42 ± 4.0 (5)	66 ± 5.5 (5)	54 ± 4.0 (5)
Pars reticulata	44 ± 4.3 (3)	34 ± 3.4 (5)	53 ± 3.0 (5)	47 ± 3.2[b] (5)
Striatum	89 ± 18 (2)	38 ± 5.0[a] (5)	91 ± 4.5 (5)	57 ± 4.9[a,b] (5)
Nucleus accumbens	91 ± 21 (2)	49 ± 5.8[a] (5)	86 ± 5.6 (5)	67 ± 8.5 (5)
Lamina plexiformis externa bulbi olfactorii	63 ± 1.5 (2)	55 ± 7.1 (5)	62 ± 6.3 (5)	49 ± 5.8 (5)
Tuberculum olfactorium	82 ± 23 (2)	47 ± 5.8 (5)	72 ± 8.4 (5)	55 ± 9.9 (5)
Thalamus				
Nucleus anterior	67 ± 4.0 (3)	48 ± 3.8 (5)	61 ± 7.8 (5)	46 ± 4.2[a] (5)
Nucleus ventralis	82 ± 4.4 (3)	45 ± 3.2[a] (5)	93 ± 12 (5)	59 ± 6.5 (5)
Nucleus ventromedialis	100 ± 8.0 (3)	47 ± 4.9[a] (5)	94 ± 12 (5)	67 ± 8.7[a] (5)
Nucleus periventricularis	63 ± 5.9 (3)	39 ± 3.7[a] (5)	62 ± 2.9 (5)	45 ± 3.8[a] (5)
Nucleus medialis	98 ± 3.2 (3)	41 ± 4.0[a] (5)	80 ± 6.1[a] (5)	56 ± 4.0[a,b] (5)
Nucleus reticularis	69 ± 8.8 (3)	37 ± 2.7[a] (5)	72 ± 7.2 (5)	48 ± 3.7[a] (5)
Nucleus reuniens	65 ± 11 (3)	41 ± 4.4[a] (5)	61 ± 4.4 (5)	49 ± 6.7 (5)
Nucleus rhomboideus	83 ± 8.4 (3)	44 ± 4.5[a] (5)	88 ± 9.3 (5)	65 ± 6.9 (5)
Nucleus posterior	74 ± 6.5 (3)	47 ± 4.8 (5)	80 ± 8.1 (5)	63 ± 7.0 (5)
Nucleus parafascicularis	80 ± 11 (3)	59 ± 3.3 (5)	87 ± 7.4 (5)	66 ± 4.8 (5)
Hippocampus				
Gyrus dentatus dorsalis	47 ± 7.1 (3)	42 ± 4.3 (5)	51 ± 4.9 (5)	40 ± 2.6 (5)
Gyrus dentatus ventralis	53 ± 5.0 (3)	43 ± 5.6 (5)	56 ± 2.8 (5)	43 ± 1.9 (5)
CA1, dorsalis	50 ± 7.7 (3)	45 ± 4.5 (5)	44 ± 4.8 (5)	39 ± 1.8 (5)
CA3, dorsalis	56 ± 11 (3)	55 ± 5.2 (5)	46 ± 3.6 (5)	42 ± 1.1 (5)
CA1, ventralis	59 ± 4.9 (3)	40 ± 3.5[a] (5)	54 ± 3.3 (5)	42 ± 2.6[a] (5)
CA2 + 3, ventralis	47 ± 3.3 (3)	40 ± 6.0 (5)	47 ± 3.7 (5)	38 ± 2.7 (5)
Cortex				
Auditiva, lamina IV	144 ± 7.7 (3)	44 ± 6.4[a] (5)	91 ± 4.5[a] (5)	65 ± 2.4[a,b] (5)
Auditiva, lamina V	114 ± 3.8 (3)	41 ± 6.3[a] (5)	73 ± 4.7[a] (5)	60 ± 3.8[a,b] (5)
Frontalis, lamina IV	99 ± 12 (2)	40 ± 2.8[a] (5)	75 ± 4.0[a] (5)	52 ± 5.5[a] (5)
Frontalis, lamina V	77 ± 11 (2)	34 ± 3.2[a] (5)	72 ± 4.7 (5)	43 ± 4.3[a] (5)
Medialis	73 ± 4.6 (3)	45 ± 6.4[a] (5)	59 ± 3.5 (5)	46 ± 2.5[a] (5)

[a] Significantly different from control, $p \leq 0.05$.

[b] Significantly different from muscimol, $p \leq 0.05$.

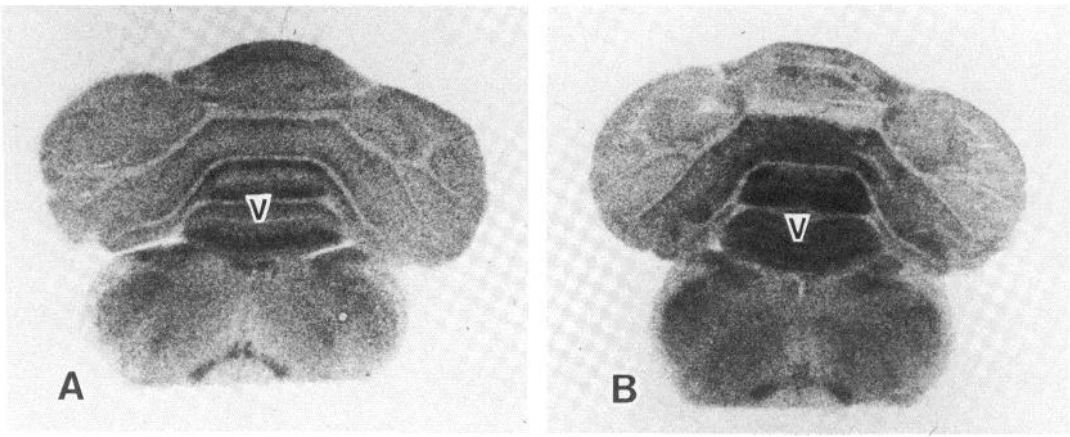
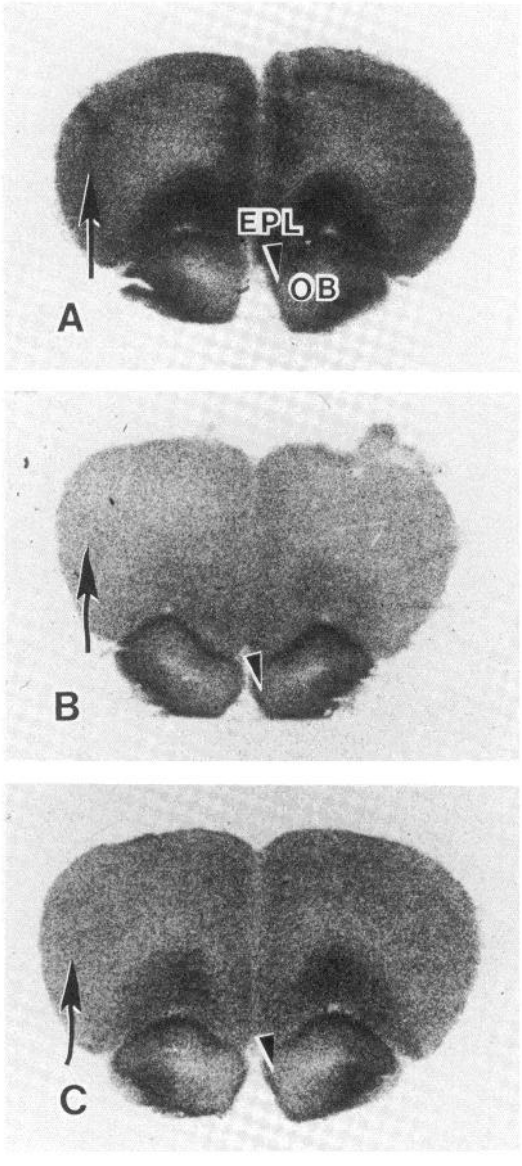


Figure 1. Effect of scopolamine on autoradiographic grain densities, representing glucose utilization, in the rat cerebellum. These are photographs of x-ray film exposed to 20-μm brain sections from a control rat (*A*) injected with 0.9%, w/v, NaCl (1 ml/kg, i.v., 30 and 14 min before [^{14}C]DG) and another rat (*B*) injected with scopolamine (2.0 mg/kg, i.v., 30 min before [^{14}C]DG) and 0.9% NaCl (14 min before [^{14}C]DG). Note the increased density in the cerebellar vermis (*V*) after scopolamine treatment. Magnification × 5.6.



mesencephalic reticular formation and produced a nonsignificant increase in the cerebellar vermis. The high dose significantly elevated LCGU in the mesencephalic reticular formation and almost doubled LCGU in the cerebellar vermis.

The effects of muscimol on LCGU reported here agree with earlier findings (Kelly and McCulloch, 1982; Palacios et al., 1982) in which the distribution of GABA agonist-induced LCGU effects did not simply correspond to the regional distribution of markers for GABAergic synapses (Chalmers et al., 1970; De Montis et al., 1981; Krogsgaard-Larsen and Falch, 1981). For example, the pars reticulata of the substantia nigra, which has one of the highest GABA concentrations in the brain, showed only a slight nonsignificant decrease in LCGU after muscimol treatment. In addition, LCGU in some regions with high levels of [^{3}H]muscimol binding, such as the molecular layer of the hippocampus, the dentate gyrus, and the external plexiform layer of the olfactory bulb (Chan-Palay, 1978; Palacios et al., 1980, 1981a), was not affected by muscimol. These observations may reflect the fact that muscimol is degraded rapidly and that only a small proportion of injected muscimol seems to reach the brain (Baraldi et al., 1979; Maggi and Enna, 1979).

Figure 2. Effects of muscimol and scopolamine + muscimol on autoradiographic grain densities, representing glucose utilization, in the rat olfactory bulb and frontal cortex. These are photographs of x-ray film exposed to 20-μm brain sections from a control rat (*A*) injected with 0.9%, w/v, NaCl (1 ml/kg, i.v., 30 and 14 min before [^{14}C]DG); a rat (*B*) treated with 0.9% NaCl (30 min before [^{14}C]DG) + muscimol (4.0 mg/kg, i.v., 14 min before [^{14}C]DG); and a rat (*C*) treated with scopolamine (0.4 mg/kg, i.v., 30 min before [^{14}C]DG) + muscimol (4.0 mg/kg, i.v., 14 min before [^{14}C]DG). Although muscimol alone was without an effect in the olfactory bulb, scopolamine + muscimol decreased labeling in the external plexiform layer (*EPL*) of the olfactory bulb (*OB*). In rats treated with muscimol alone or with scopolamine + muscimol, labeling of layers IV and V of the frontal cortex was reduced. Magnification × 5.6.

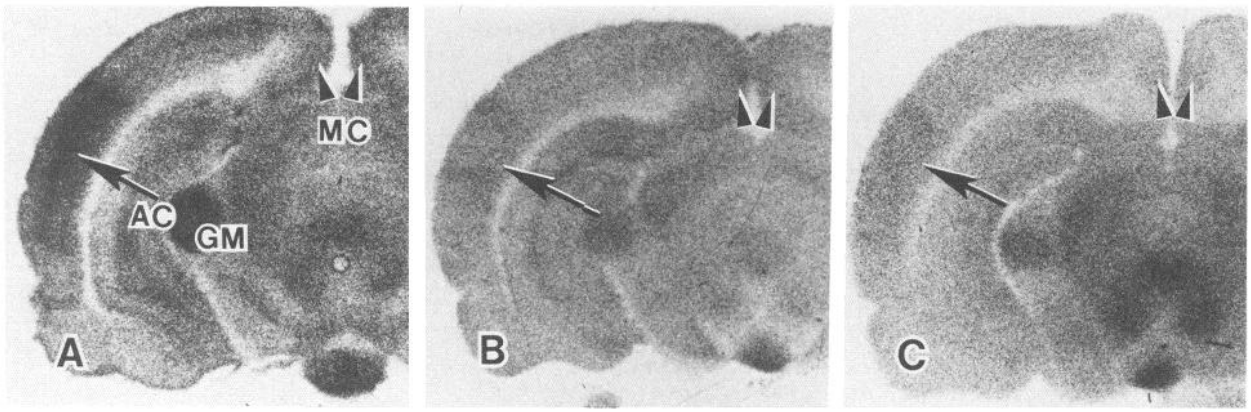


Figure 3. Effects of muscimol and scopolamine + muscimol on autoradiographic grain densities, representing glucose utilization, in the rat cerebral cortex. These are photographs of x-ray film exposed to 20-μm brain sections from a control rat (*A*) injected with 0.9%, w/v, NaCl (1 ml/kg, i.v., 30 and 14 min before [^{14}C]DG); a rat (*B*) injected with 0.9% NaCl (30 min before [^{14}C]DG) and muscimol (4.0 mg/kg., i.v., 14 min before [^{14}C]DG); and a rat (*C*) injected with scopolamine (0.4 mg/kg, i.v., 30 min before [^{14}C]DG) and muscimol (4.0 mg/kg, i.v., 14 min before [^{14}C]DG). Although muscimol decreased labeling in the medial cortex (*MC*) and auditory cortex (*AC*), scopolamine pretreatment further decreased labeling in both areas. *GM*, medial geniculate body. Magnification × 5.6.

Furthermore, as in all CNS systems, neural circuitry influences the metabolic responses to GABAerigic drugs (Palacios et al., 1982). Thus, in a particular region, LCGU following systemic agonist treatment has contributions from cells receptive to the agonist as well as from projections which may not respond to the agonist; this latter contribution would tend to dilute specific agonist effects in that brain region. In addition, the distribution of metabolic responses to a specific agonist would reflect, to some extent, primary drug-receptor interactions in various loci as well as secondary effects propagated to other brain regions by way of efferents.

In some brain regions, scopolamine enhanced a muscimol-induced decrease in LCGU. In the anterior, periventricular, reticular, and parafascicular thalamic nuclei, where neither muscimol nor scopolamine alone affected LCGU, the combined treatment with the lower dose of scopolamine (0.4 mg/kg) and the higher dose of muscimol (4.0 mg/kg) was associated with significant LCGU decreases. Furthermore, in the medial cortex and nucleus accumbens, where muscimol alone significantly reduced LCGU, scopolamine pretreatment enhanced the muscimol-induced decreases. The LCGU effects in the medial cortex and nucleus accumbens may, in part, reflect the combined drug effect on thalamic structures from which these areas receive afferents (Newman, 1980; Domesick, 1981). These data suggest that GABA and GABAmimetic drugs might antagonize cholinergic transmission in some pathways, perhaps by inhibiting cholinergic or cholinoceptive neurons.

Among those brain regions where an antagonistic GABAergic-cholinergic interaction was manifested, the reticular nucleus and the parafascicular nucleus of the thalamus have been implicated in analgesic and/or nociceptive mechanisms. Electrophysiological data suggest that the reticular thalamic nucleus has inhibitory control over cutaneous and noxious mechanical stimuli (Peschanski et al., 1980). Electrical stimulation of the spinothalamic tract, which carries nociceptive information, changes the firing rates of the parafascicular thalamic nucleus, supporting the concept of a role for this nucleus in coding for nociceptive input (Ryu et al., 1979; McClung and Dafny, 1980). Therefore, LCGU in some brain regions related to nociception shows an apparent GABAmimetic-cholinergic antagonism.

In other brain regions, a high dose of scopolamine (2.0 mg/kg) blocked the LCGU decreases produced by 1.6 mg/kg of muscimol. This occurred in the central gray matter, locus ceruleus, and ventral thalamic nucleus, which are brain regions typically associated with analgesia (Mayer and Price, 1976; Behbehani and Fields, 1979; Bogduk and Lance, 1981) and which have high opiate receptor densities (Pert et al., 1976). This observation implies that cholinergic neurotransmission is required, in some cases, for the expression of muscimol effects on LCGU, and it supports previous evidence for a cholinergic link in GABAergic antinociception (Kendall et al., 1982). Furthermore, the present findings suggest that the reported antagonism by atropine of GABAmimetic-induced antinociception (Kendall et al., 1982) has a metabolic correlate in brain regions including the central gray matter, locus ceruleus, and ventral thalamic nucleus.

Although it appears that the interactions described here may represent *in vivo* GABA-acetylcholine links, this interpretation is limited by several factors. For example, it is not certain that all muscimol effects are GABAergic. In this regard, systematically administered muscimol is degraded rapidly, and many effects of muscimol might be due to metabolites (Baraldi et al., 1979; Maggi and Enna, 1979). On the other hand, muscimol effects on LCGU resemble those of 4,5,6,7-tetrahydroisoxazolo[5,4-C]pyridin-3-ol (THIP); and muscimol-induced LCGU decreases can be antagonized at least partially by bicuculline (Palacios et al., 1982). Another complication is the fact that GABA can produce inhibitory electrophysiological responses even in neurons with no known inhibitory input (Kuffler and Edwards, 1958). Thus, even direct effects of GABA receptor agonists could occur in brain regions which normally do not

participate in GABAergic transmission. Therefore, additional studies employing drugs which more selectively activate GABAergic synapses by other mechanisms, such as inhibition of GABA uptake, would be of value. The present findings with muscimol can be useful in directing such efforts.

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