Pharmacology Biochemistry & Behavior, Vol. 32, pp. 1067–1070,© Maxwell Pergamon Macmillan plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

BRIEF COMMUNICATION

Intranigral GABAergic Drug Effects on Striatal Dopamine Activity

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Received 5 May 1988

SPERBER, E. F., J. N. D. WURPEL, N. S. SHARPLESS AND S. L. MOSHÉ. *Intranigral GABAergic drug effects on striatal dopamine activity*. PHARMACOL BIOCHEM BEHAV **32**(4) 1067–1070, 1989.—Concentrations of striatal dopamine (DA), serotonin (5-HT) and their metabolites were measured following infusions of the $GABA_A$ receptor agonist, muscimol, or $GABA_A$ receptor antagonist, bicuculline, into the substantia nigra (SN) or areas dorsal to the SN in adult rats and 16-day-old rat pups. Results indicated that intranigral infusions of muscimol produced site-specific increases in the concentrations of striatal DA metabolites in adults, while in pups, intranigral muscimol infusions produced site-specific increases in the concentrations of striatal DA. Intranigral infusions of bicuculline had no effect on striatal DA or its metabolites in either age group. Neither GABAergic drug had any effect on striatal 5-HT or its metabolite. The data suggest that the effect of nigral $GABA_A$ agonist infusions on the activity of the nigrostriatal pathway is age-specific. The lack of opposing effects following the nigral infusion of a $GABA_A$ receptor antagonist indicates that the influences of $GABA_A$ agonists may be mediated by different mechanisms as a function of age.

GABA Substantia nigra Striatum Dopamine Rat Animal newborn Seizures

PRELIMINARY data suggest that infusions of muscimol into the substantia nigra (SN) alter the activity of one of the main nigral efferent systems, the nigrostriatal pathway, as a function of age (12). We observed that nigral muscimol infusions in adult rats increase striatal dopamine (DA) utilization while in pups, nigral muscimol infusions decrease striatal DA utilization. These age-related striatal changes parallel the age-related effects of nigral muscimol infusions on seizures, i.e., suppression of seizures in adult rats (4, 5, 7, 9, 17) and facilitation in pups (10, 14, 16). To further clarify the role of the GABA-sensitive nigrostriatal pathway in seizure modification, the present study was designed to compare the effects of nigral infusions of a GABA agonist, muscimol, and antagonist, bicuculline, on the activity of striatal DA, serotonin (5-HT) and their metabolites in adult rats and rat pups. The site specificity of these effects were studied by infusing muscimol and bicuculline dorsal to the SN and by determining the amount of muscimol spread.

METHOD

To evaluate whether the effects of the nigral infusions were due to differences in the amount of drug distribution from the infusion site, the spread of ^{3}H-muscimol was determined by liquid scintillation spectroscopy of brain areas obtained by two methods: cryostat derived sections or microdissected brain areas. Sprague-Dawley albino adult male rats (220–250 g) and 14-day-old pups (the date of birth counted as day 0) had a cannula (Plastic Products) unilaterally implanted into the left SN (see surgical procedure below). Following a two-day recovery period, 100 ng of ^{3}H-muscimol (300,000–350,000 cpm) in 0.25 μl saline was injected intranigrally over a 4-min period. The consistency and accuracy of the infusion was controlled for by using teflon tubing (Small Parts Inc.) to reduce the compliance of the infusion apparatus. The tubing was connected to a 33-gauge internal cannula (Plastic Products) at one end and a microsyringe (Hamilton Co.) at the other. In all experiments, the constancy of the infused volume was demonstrated by comparing ^{3}H-muscimol scintillation counting of 0.25 μl samples from the infusion system to the stock radioligand solution. At 30 min postinfusion, the rats (n=4 pups, n=3 adults) were decapitated and the brains were rapidly removed, frozen in liquid freon chilled to −35°C and later cut into 20 μ sections. Alternating sections were either stained with thionin for histological verification or placed in scintillation vials for tritium counting. In the microdisection procedure, the rats were decapitated 1 min (n=4 pups, n=3 adults) or 30 min (n=4 pups, n=5 adults) after the ^{3}H-muscimol infusion. The brains

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were rapidly removed and placed on ice and the following areas were dissected ipsilaterally and contralaterally: SN, dorsal to the SN area, striatum, olfactory bulb, pons and the cerebellum. For each brain region, the tissue was weighed and dissolved in 1 ml of 1.0 NaOH at 40°C overnight. Six ml of ACS liquid scintillation cocktail were added and the samples were counted for tritium. The cpm for the digested brain regions were expressed as percent values of the control injection (0.25 μl of ^{3}H-muscimol), as well as cpm/mg wet weight of tissues.

To study the effect of infusions of muscimol and bicuculline in or dorsal to the SN on striatal biogenic amine concentrations, a group of Sprague-Dawley albino adult male rats (275–300 g) and 14-day-old pups had cannulae implanted into the SNR (pars reticulata of the SN) bilaterally. The adult rats were anesthetized with a mixture of ketamine 70 mg/kg and xylazine 10 mg/kg, IM, while the pups with ketamine 66 mg/kg IP. The stereotaxic coordinates for the adult SNR were: 5.3 mm posterior and 4.0 mm lateral to bregma, 7.7 mm deep at an angle of 15°; and for the pups: 5.2 mm posterior and 3.5 mm lateral to bregma, 6.5 mm deep from the skull at an angle of 15°. The incisor bar was 3.5 mm below the horizontal plane. An additional group of adult and rat pups had cannulae bilaterally implanted (approximately 2 mm) dorsal to the SN (in the midbrain reticular formation) to serve as a control and to determine the site specificity of the observed effects. The stereotaxic coordinates for this group were the same as described above with the exception of the depth placement. The cannula was lowered for the pup: 4.7 mm from the skull and for the adult: 5.4 mm. Two days following surgery, the rats were infused with either muscimol (100 ng/0.25 μl per site), bicuculline methobromide (100 ng/0.25 μl per site), or an equivalent volume of saline. These doses were selected since they produce maximal age-related effects on seizures (16,17). The drugs were dissolved in saline and infused over a period of 2 min per site via a 33-gauge internal cannula.

The rats were observed for 30 min after the completion of the muscimol infusion and for 5 min after the bicuculline infusion. The times were selected based on previous studies documenting the effects of the drugs on seizures (7, 9, 10, 14, 16). All experimental animals were observed to engage in a variety of stereotypic behaviors including excessive sniffing, licking, head movements, some circling, grooming and wet dog shakes. At the end of the observation period, the rats were decapitated, the brains were removed and placed on their dorsal surface on ice. A perpendicular cut was made posterior to the olfactory tubercles. The left and right striatum was removed from the rostral piece and analyzed separately.The tissues were immediately frozen on dry ice and later analyzed for DA, 5-HT and their metabolites: 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) or 5-hydroxyindoleacetic acid (5-HIAA) using high-performance liquid chromatography with electrochemical detection (column: Biophase ODS, 5 μm from Bioanalytical Systems; mobile phase: 0.1 M chloracetate, pH 3.2, 1 mM Na_2EDTA, 0.20 mM sodium octylsulfate and 8% methanol at 1.0 ml/min). The frozen tissues were weighed, homogenized in ice-cold 0.1 N $HClO_4$ and centrifuged. An aliquot of the clear supernatant fluid was injected directly onto the column. Quantitation was performed by determination of peak heights relative to the heights of external standards. The caudal portion of the brain was frozen in freon and liquid nitrogen. Using standard techniques, 20 μ sections were obtained for histological verification of the cannulae placement. Striatal concentrations of DA, 5-HT and their metabolites were analyzed only if the ipsilateral cannula placement was either in the SN or dorsal to the SN. The data were analyzed statistically using the one-way analysis of variance with Neuman-Keuls post hoc comparisons.

RESULTS

Drug Distribution

The spread of muscimol was minimal. Using the microdissection technique, we found that at 1 min postinfusion, the muscimol spread was similar in both the adult and pup with $8.3 \pm 0.9\%$ remaining in the pup SN and $8.1 \pm 0.5\%$ remaining in the adult SN. For the most part, there was no significant spread into adjacent areas (dorsal to the SN) or more distant areas (contralateral SN, striatum, olfactory bulb or cerebellum). At 30 min postinfusion, 0.5% of the original ^{3}H-muscimol concentrations remained in the SN. Analysis of the tritium content of the serially obtained sections revealed that in both age groups, at 30 min postinfusion, the ^{3}H-muscimol spread encompassed an area (anterior-posterior) of 2.5 to 1.5 mm from the site of injection. At these extremes, the concentration of muscimol was 10% of that found at the site of injection.

Striatal Effects

In the adult rats, microinfusions of nigral muscimol resulted in a significant increase in striatal concentrations of DOPAC, $F(2,51) = 4.49$, $p<0.02$, and HVA, $F(2,49)=4.37$, $p<0.02$, while the DA concentrations did not differ from the saline infused group (Fig. 1). The effect appears to be specific to DA and its metabolites since striatal concentrations of 5-HT and its metabolite, 5-HIAA, were unaffected. Microinfusions of muscimol into the SN produced different results in the 16-day-old pup. The DA concentrations were markedly elevated, $F(2,49)=13.87$, $p<0.001$, while the concentrations of DOPAC and HVA remained unaffected (Fig. 2). These effects also appear to be specific for DA and its metabolites since muscimol had no effect on the concentrations of 5-HT and its metabolite, 5-HIAA. In contrast to the effects produced by nigral muscimol infusions, nigral bicuculline infusions did not have any effect on striatal concentrations of DA, 5-HT or their metabolites in either adults (Fig. 1) or pups (Fig. 2).

In the adult rat, microinfusions of muscimol or bicuculline above the SN had no effect on striatal concentrations of DA, 5-HT or their respective metabolites. However, in the pups, infusions of muscimol dorsal to the SN increased the concentrations of the DA metabolites, DOPAC, $F(2,31)=3.86$, $p<0.03$, and HVA, $F(2,31)=3.63$, $p<0.04$, while bicuculline infusions dorsal to the SN had no significant effect.

DISCUSSION

The spread study suggests that muscimol remains relatively localized within the SN to both age groups. Our data demonstrate that at 30 min postinfusion, the concentrations of muscimol is much less than the amount infused and is consistent with that reported by others (13). These findings reconcile the discrepancy between the large doses that are used for localized pharmacological studies and the small amounts required for receptor activation. Our findings indicate that at 30 min postinfusion, the concentration of nigral muscimol is approximately 5 pmol which is well within the range of physiological concentrations (3).

In adult rats, intranigral muscimol infusions resulted in an increase in striatal DA utilization as indicated by the observed increases in DOPAC and HVA levels. In contrast, in pups, intranigral muscimol infusions increased striatal DA levels, but had no effect on the levels of striatal DA metabolites. Intranigral infusions of bicuculline did not affect the striatal concentrations of DA and its metabolites in either age group.

The results from adult rats are consistent with those of Wood (19) and Kilpatrick *et al.* (8) in which it was reported that parenteral and intranigral muscimol injections had no effect on DA

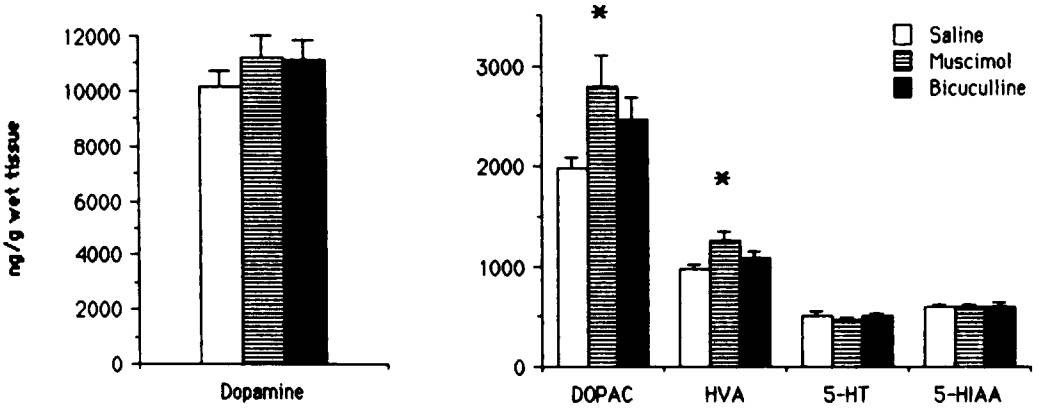

FIG. 1. Effects of intranigral infusions of muscimol (100 ng/0.25 μl, per site) or bicuculline (100 ng/0.25 μl, per site) on striatal concentrations of DA, 5-HT and their metabolites in adult rats. The values are means ± S.E.M. in ng/g of wet tissue and depict biogenic amine concentrations from only those striata with an accurate ipsilateral SNR placement. The left and right striatal tissue was analyzed separately. Intranigral infusions of muscimol (n = 16) increased concentrations of DA metabolites (DOPAC and HVA) in adult striatum ($p<0.02$), while intranigral infusions of bicuculline (n = 15) had no effect when compared to rats treated with saline (n = 24).

levels while producing a significant increase in DOPAC and HVA levels in the striatum. Bicuculline had no effect on striatal DA, DOPAC or HVA levels when measured at 30 min following drug treatment. On the other hand, Waddington and Cross (18) reported that intranigral muscimol increased both striatal DA and DOPAC levels without affecting 5-HT or HVA levels.

In the present study, stereotypic behaviors were observed both in adult and rat pups following nigral muscimol and bicuculline infusions. The stereotypies appeared to be identical in the two age groups although the effect of these drugs on striatal catecholaminergic concentrations were not the same. These findings suggest that the stereotypic behaviors induced by GABAergic drugs are not necessarily dependent on the nigrostriatal pathway and DA levels. While others have reported GABA-related stereotypies to be independent of the nigrostriatal pathway in adults (2), this has not been demonstrated in pups till now.

Both muscimol and bicuculline have a high affinity for the $GABA_A$ receptor subtype; muscimol acts as an agonist while bicuculline blocks the receptor (6). In adult rats, the results of pharmacological studies indicate that nigral muscimol and bicuculline produce opposite effects on seizures, that is, nigral muscimol suppresses seizures (4, 5, 7, 9, 17), while nigral bicuculline facilitates the onset of seizures (17). These observations suggest that the nigral $GABA_A$ receptor subtype has an important role in the regulation of seizures of adult rats. The pathways that mediate the nigral effects on seizures have not been completely elucidated (14). If the nigral effects on seizures were to be mediated by the striatum, we would have expected to find that nigral infusions of muscimol and bicuculline (in doses that modify seizures) would produce the opposite effect on striatal biogenic amine concentrations. This was not the case. Therefore, our data do not support the striatal involvement and are in agreement with

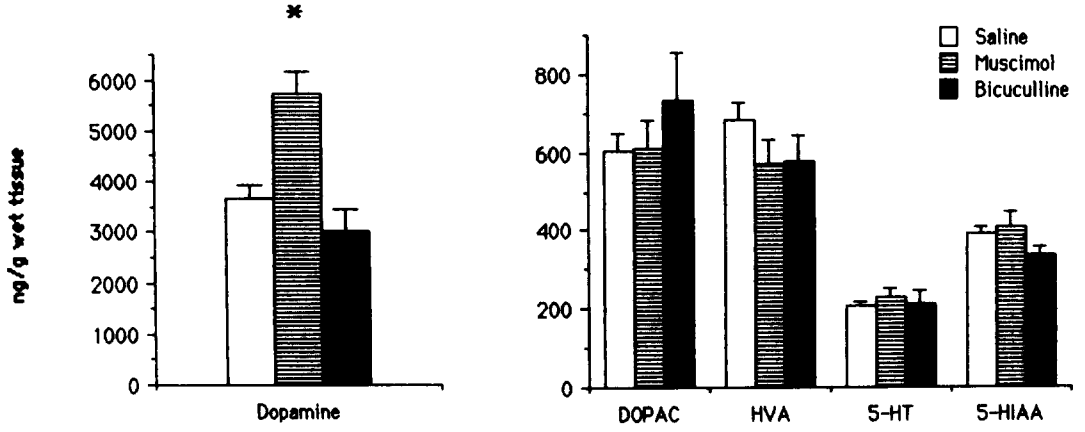

FIG. 2. Effects of intranigral infusions of muscimol (100 ng/0.25 μl, per site) or bicuculline (100 ng/0.25 μl, per site) on striatal concentrations of DA, 5-HT and their metabolites in rat pups. The values are means ± S.E.M.in ng/g of wet tissue and depict biogenic amine concentrations from only those striata with an accurate ipsilateral SNR placement. The left and right striatal tissue was analyzed separately. Intranigral infusions of muscimol (n = 16) increased concentrations of DA in the pup striatum ($p<0.001$), while intranigral infusions of bicuculline (n = 10) had no effect when compared to rats treated with saline (n = 20).

previous studies in which lesions of the SN pars compacta failed to alter the susceptibility to kindling [(1,15), Wasterlain, personal communication].

In rat pups, the pathways involved in the mediation of the nigral effects on seizures may be different from adults (14). Thus, in pups, the effects of muscimol on striatal DA may be significant in demonstrating a possible role of the striatum on seizures in this age group. We have shown that bicuculline and muscimol do not produce opposite effects on seizures (21), therefore, it is not necessary to presume that they would produce opposite striatal effects. In fact, the difference in action between the two drugs suggests that their proconvulsant effects on seizures may be mediated by different pathways.

ACKNOWLEDGEMENTS

This paper is dedicated to the memory of Dr. N. S. Sharpless. We thank Ms. M. Downing for her technical assistance and Ms. D. Platyan for her secretarial assistance. Supported in part by Grants NS 20253 from the NINCDS and R-36986 from the United Cerebral Palsy Associations.

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