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European Journal of Pharmacology 299 (1996) 93–102

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Neither intranigral fluoxetine nor 5,7-dihydroxytryptamine alter audiogenic seizures in genetically epilepsy-prone rats

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Received 10 August 1995; revised 28 November 1995; accepted 5 December 1995

Abstract

Previous studies have shown that widespread depletion of brain 5-hydroxytryptamine (5-HT, serotonin) exacerbates audiogenic seizures in genetically epilepsy-prone rats (GEPRs), while elevations in brain 5-HT attenuate these seizures. However, the location of the central nervous system site(s) at which 5-HT exerts its anticonvulsant action on audiogenic seizures, remains unknown. The substantia nigra has been shown to exert modulatory actions over both brainstem and forebrain driven seizures in normal rats, and receives a rich serotonergic innervation. The present study was designed to determine if 5-HT exerts its modulatory effect on audiogenic seizures by an action in the substantia nigra. Microinfusion of 5,7-dihydroxytryptamine (4 μg/0.25 μl bilateral) into the substantia nigra of GEPRs which display a moderate seizure (GEPR-3s) failed to alter the audiogenic seizure. Consistent with these findings, microinfusions of fluoxetine-HCl into the substantia nigra of severe seizure GEPRs (GEPR-9s) failed to alter any aspect of the audiogenic seizure. This effect was observed when fluoxetine was infused alone, or in combination with systemic administration of 5-hydroxytryptophan (75 mg/kg, i.p.). The present findings argue against a modulatory role of nigral 5-HT on audiogenic seizures in GEPRs.

Keywords: Seizure; 5-HT (5-hydroxytryptamine, serotonin); Fluoxetine; Genetically epilepsy-prone rat; Substantia nigra

1. Introduction

Previous studies have provided evidence implicating 5-hydroxytryptamine (5-HT, serotonin) as a modulator of convulsive seizures. In general, a negative correlation between brain 5-HT levels and seizure severity has been observed using a variety of seizure models including: electroshock (Buterbaugh, 1977, 1978; Browning et al., 1978; Burley and Ferrendelli, 1984; Löscher et al., 1984), systemically administered pentylenetetrazole or lidocaine (De La Torre et al., 1970; Kovacs and Zoll, 1974; Burley and Ferrendelli, 1984; Lazarova et al., 1983; Löscher and Czuczwar, 1985; Endo et al., 1993), neocortical and hippocampal kindling (Racine and Coscina, 1979; Wada et al., 1991, 1993), and injections of bicuculline into the deep prepiriform cortex/area tempestas (Pasini et al., 1992; Prendiville and Gale, 1993). Moreover, increases in brain 5-HT attenuate seizures in numerous genetic models of convulsive seizures, including the photosensitive baboon (*Papio papio*) (Wada et al., 1972; Meldrum et al., 1972), the El mouse (Hiramatsu, 1981), and the genetically epilepsy-prone rat (Jobe et al., 1973a; Dailey et al., 1992a; Yan et al., 1992).

The genetically epilepsy-prone rat (GEPR) is a widely used model of inherited generalized tonic-clonic epilepsy. Today two strains of GEPR exist: a moderate-seizure (GEPR-3) strain and severe-seizure (GEPR-9) strain (Dailey et al., 1989). In addition to being susceptible to audiogenic seizures, GEPRs are uniquely susceptible to seizures induced by hyperthermia (a characteristic retained in adulthood) and a significant incidence of spontaneous seizures has been observed in the GEPR colonies (Jobe et al., 1986; Dailey et al., 1989). The GEPR also exhibits a predisposition to seizures induced by electroshock, pentylenetetrazole, kindling and high atmospheric pressure (Reigel et al., 1986; Browning et al., 1990; Laird and Jobe, 1987; Dailey et al., 1989; Millan et al., 1991).

Several studies have shown that the severity of audiogenic seizures in GEPRs is inversely related to brain 5-HT levels. Systemic administration of *p*-chlorophenylalanine or intracerebroventricular (i.c.v.) injection of 5,7-dihydroxytryptamine was found to produce widespread and

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SSDI 0014-2999(95)00839-X

selective depletion of brain 5-HT, and exacerbate audiogenic seizures in GEPRs (Jobe et al., 1973a; Statnick et al., 1992). Moreover, administration of the 5-HT precursor, 5-hydroxytryptophan, in combination with a monoamine oxidase inhibitor was found to attenuate audiogenic seizures in GEPRs (Jobe et al., 1973a). Similarly, administration of 5-HTP in combination with fluoxetine was found greatly to increase the anticonvulsant effect produced by either agent given alone and greatly to increase the extracellular concentration of 5-HT as measured by microdialysis (Yan et al., 1994a, b). More recently, the time course for the anticonvulsant effect of carbamazepine and fluoxetine was found to correlate with an increase in extracellular brain 5-HT in both GEPR-3s and GEPR-9s (Dailey et al., 1992a; Yan et al., 1992) and depletion of brain 5-HT was found to attenuate the anticonvulsant effect produced by these agents (Yan et al., 1992, 1994a).

Despite evidence that 5-HT exerts an anticonvulsant effect in GEPRs, the site(s) of this effect has not been identified. However, there is considerable support for the hypothesis that the substantia nigra is part of an endogenous system that exerts a modulatory role on seizure activity in the rat (Gale, 1992). For example, focal injections of GABA receptor agonists into the substantia nigra pars reticulata have been shown to inhibit seizures induced by pilocarpine, flurothyl, maximal electroshock, kindling, pentylenetetrazole, or focal injections of bicuculline into the area tempestas (Iadarola and Gale, 1982; Turski et al., 1990; Xu et al., 1991; Zhang et al., 1991). More recently, intranigral administration of fluoxetine, a 5-HT reuptake inhibitor, was shown to inhibit limbic motor seizures induced by focal injections of bicuculline into the area tempestas, an effect that is believed to be mediated via an increase in 5-HT neurotransmission (Pasini et al., 1992). Inasmuch as elevations in nigral 5-HT appear to reduce the intensity of a forebrain driven seizure (i.e., limbic motor), it was of interest to determine if nigral 5-HT also modulates audiogenic seizures, which are brainstem driven convulsions (Browning et al., 1991a). Thus, the present study was designed to determine if audiogenic seizures can be modified by alterations in 5-HT neurotransmission within the substantia nigra. This was accomplished by assessing the effects of removing 5-HT from the substantia nigra via a focal infusion of 5,7-dihydroxytryptamine in GEPR-3s, and by facilitating 5-HT neurotransmission in the substantia nigra of GEPR-9s through focal injections of fluoxetine. GEPR-3s, which display a moderate clonic convulsion in response to sound stimulation were employed for the 5-HT depletion study because this treatment was expected to increase seizure severity, which is more easily detected in GEPR-3s. Indeed, an increase in seizure severity is difficult to detect in GEPR-9s because they display a maximal tonic extensor convulsion in response to sound stimulation. On the other hand, GEPR-9s were employed in studies where the treatment was expected to reduce seizure severity because reductions in severity are more easily detected in GEPR-9s. The use of both GEPR-3s and GEPR-9s was justified because both have widespread deficits in brain 5-HT concentration (Dailey et al., 1992b) and pharmacological modification of serotonergic neurotransmission have analogous effects on seizure severity in both strains of GEPR (Jobe et al., 1973a; Statnick et al., 1992; Yan et al., 1994a, b). The latter findings also suggest that the serotonergic mechanism responsible for regulating seizure severity is common to both strains of GEPR.

2. Materials and methods

2.1. Audiogenic seizure testing

To produce an audiogenic seizure, GEPRs were placed in a cylindrical Plexiglas chamber (40 cm in diameter × 50 cm in height) with a lid equipped with two door bells which deliver approximately 100 db of mixed frequency sound. The stimulus was initiated within 30 s after the rat was placed in the chamber, and continued until the onset of convulsion or for a maximum of 60 s. The latency to the onset of wild running and the latency to the onset of convulsion (whether tonic or clonic) were recorded. The seizure severity was also scored using the system described by Jobe et al. (1973b). This severity scoring system assigns scores ranging from 0 to 9: with 0 = no response, 1 = wild running only, 2 = two running phases ending in a clonic convulsion, 3 = one running phase ending in a clonic convulsion, 4 = two running phases ending in tonic extension of the forelimbs, 5 = one running phase ending in tonic extension of the forelimbs, 6 = two running phases ending in nearly complete tonic extension of the hindlimbs, 7 = one running phase ending in nearly complete tonic extension of the hindlimbs, 8 = two running phases ending in a complete tonic extensor convulsion and 9 = one running phase ending in a complete tonic extensor convulsion. All rats received two to three pretests at least 48 h apart prior to any experiment. Rats displaying inconsistent severity scores during the pretests were eliminated from the study.

2.2. Infusions of 5,7-dihydroxytryptamine into the substantia nigra of GEPR-3s

Following the three audiogenic seizure pretests (at least 48 h apart), male GEPR-3s (307–464 g) were treated with protriptyline (20 mg/kg, i.p.) to protect catecholaminergic neurons from the neurotoxic effects of 5,7-dihydroxytryptamine. After 2 h, the rats were anesthetized with chloral hydrate (400 mg/kg, i.p.), and treated with procaine penicillin (10000 IU, i.m.) and atropine sulfate (0.542 mg/kg, s.c.) to prevent postsurgical infection and to minimize respiratory congestion, respectively. The animals were then placed in a Kopf stereotaxic apparatus, and bilateral injections of 5,7-dihydroxytryptamine (4 μg/0.25

μl) were made into the substantia nigra pars reticulata using a 1 μl Hamilton syringe over a 2 min period. After each injection, the needle was left in place for 5 min before removal. The following coordinates were used for the injections: incisor bar = +5.0 mm, A.P. = −3.3 mm (from bregma), lateral = ±2.2 mm (from the midline), ventral = −7.8 mm (from dura). The 5,7-dihydroxytryptamine was made fresh each day by dissolving it in a 0.1% solution of ascorbic acid in saline, which had been bubbled with nitrogen. Control injections were made on the same day by injecting 0.25 μl of vehicle using identical coordinates. Following a 2 week postoperative period, all rats received audiogenic seizure tests at 2, 3 and 4 weeks following stereotaxic microinjection of 5,7-dihydroxytryptamine. Seizure severity scores and the latencies to wild running and to the convulsion were recorded.

2.3. Analysis of brain monoamine concentration

Following the last seizure test, rats were killed by decapitation, their brains were quickly removed, and the striatum, hypothalamus, substantia nigra, midbrain and pons-medulla were dissected out on an ice-cold plate. The tissue samples were then immediately frozen on dry ice, and stored in liquid N_2 until assayed for 5-HT and/or catecholamine content by reverse phase high performance liquid chromatography (HPLC) with electrochemical detection.

Tissue samples from both 5,7-dihydroxytryptamine and saline treated GEPR-3s were weighed and homogenized in 5 ml perchloric acid (0.2 N) in tubes containing 40 μl of methyl-5-HT (20 ng/ml final concentration) which served as an internal standard. The homogenates were centrifuged at 13000 × *g* for 2 min at 25°C. On the same day, 5-HT was separated from the other supernatant constituents (50 μl/sample) by reverse phase high performance liquid chromatography and was measured with electrochemical detection according to the method of Mefford (1981). The system employed was a Bioanalytic Systems high performance liquid chromatography with a Beckman model 110-A solvent pump, and consisted of an Ultrasphere ODS C-18 5 μm, 4.6 × 150 mm, column (Beckman) with a BAS LC-4B electrochemical detector. The detector potential was set at 650 mV vs. a Ag/AgCl reference electrode and the sensitivity was set at 10 nA/V. The mobile phase consisted of 0.1 M sodium acetate, 0.1 M citric acid and 10% (v/v) methanol at pH 4.7, which was delivered at a flow rate of 1.5 ml/min. Levels of 5-HT were calculated by comparison to standards of known concentration, and then corrected for recovery using methyl-5-HT.

A 4 ml aliquot of the remaining homogenate was taken for catecholamine assay according to the method of Browning et al. (1991b). In this procedure, 3,4-dihydroxybenzylamine was added to the homogenate (50 ng/ml final concentration) to serve as the internal standard. The homogenates were then centrifuged at 10000 × *g* for 10 min at 4°C using a Sorvall RC5B centrifuge. An aliquot of each sample (2 ml) was added to screw-top 15 ml centrifuge tubes containing 50 mg acid washed aluminum oxide, 10 μl of sodium metabisulfate solution (54.7 mM) and 10 ml of Tris-HCl buffer (0.5 M at pH 8.6). The tubes were shaken for 15 min in an Eberbach shaker (at high speed) and centrifuged at 2500 rpm for 10 min. The resulting supernatants were aspirated and the alumina was washed by shaking with 1 ml of Tris-HCl buffer (0.1 M at pH 7.0). After centrifugation and aspiration of Tris-HCl buffer, catecholamines were eluted from the alumina with 0.5 ml perchloric acid (0.2 N) . The resulting samples (eluates) were refrigerated overnight. On the following day, catecholamines were separated (using 50 μl/sample) by reverse phase high performance liquid chromatography and measured with electrochemical detection using the high performance liquid chromatography system described above. The detector potential was set at 460 mV vs. a Ag/AgCl reference electrode with a 10 nA/V sensitivity. The mobile phase contained citric acid (13 mM), Na_2HPO_4 (6.7 mM), octane sulfonate (100 μM), EDTA (50 μM) and 3% v/v methanol and was delivered at a rate of 1.5 ml/min. Brain levels of catecholamines were calculated by comparison to standards of known concentration, and corrected for recovery using dihydroxybenzylamine.

2.4. Implantation of guide cannulas for intranigral fluoxetine microinfusions

Male GEPR-9s (380–450 g) were pretested for audiogenic seizure severity 1 week prior to stereotaxic implantation of guide cannulas directed toward the substantia nigra pars reticulata. Rats were prepared for stereotaxic surgery as described above. The preliminary coordinates for cannula placement directed at the substantia nigra pars reticulata were obtained from the atlas of Pellegrino et al. (1979), and were confirmed by histology following dye injections in test animals. The following coordinates were used for all implantations: incisor bar = +5.0 mm, A.P. = −3.5 mm (from bregma), lateral = ±2.2 mm, and ventral = −7.2 mm (from dura). Guide cannulas (10 mm 23 ga stainless steel tubing) were positioned bilaterally 1 mm above the target through holes drilled in the skull, and were fixed in place with dental acrylic and jewelers screws. Stainless steel stylets (cut to the exact length of the cannulas) were placed in each cannula after surgery, to maintain cannula patency. Rats were tested postsurgically for audiogenic seizure severity following a 7–10 day recuperative period. Rats displaying severity scores that deviated from the pretests were eliminated from the study.

2.5. Intranigral microinfusion of fluoxetine in GEPR-9s

Microinfusions of fluoxetine-HCl were made alone or following pretreatment with the 5-HT precursor D,L-5-hydroxytryptophan (5-HTP) to acutely elevate brain 5-HT

levels. In those animals receiving the combination treatment, 5-HTP (75 mg/kg, i.p.) was administered 30 min prior to seizure testing. A preliminary study showed that the latter dose of 5-HTP alone did not alter audiogenic seizures in GEPR-9s. Microinfusions of fluoxetine-HCl (3.5, 7.2, and 14.1 nmol) into the substantia nigra (0.5 μl/side) were made over a 2 min period using a Harvard infusion pump. A 30 gauge infusion needle (which extended 1 mm beyond the guide cannula) was inserted into the implanted guide cannula while the rat was gently restrained. Following each infusion, the needle was left in place for 1 min. Animals were subjected to audiogenic seizure testing 15 min after infusions of fluoxetine (i.e., 30 min following treatment with 5-HTP in those animals receiving the combination) according to the previously mentioned procedure. The latencies to wild running and to the convulsion, as well as the seizure severity scores were recorded for each individual. The experiment was controlled by counterbalancing infusions of fluoxetine with 0.5 μl infusions of saline (spaced 1 week apart) in rats pretreated with either saline (i.p.) or D,L-5-HTP (75 mg/kg, i.p.).

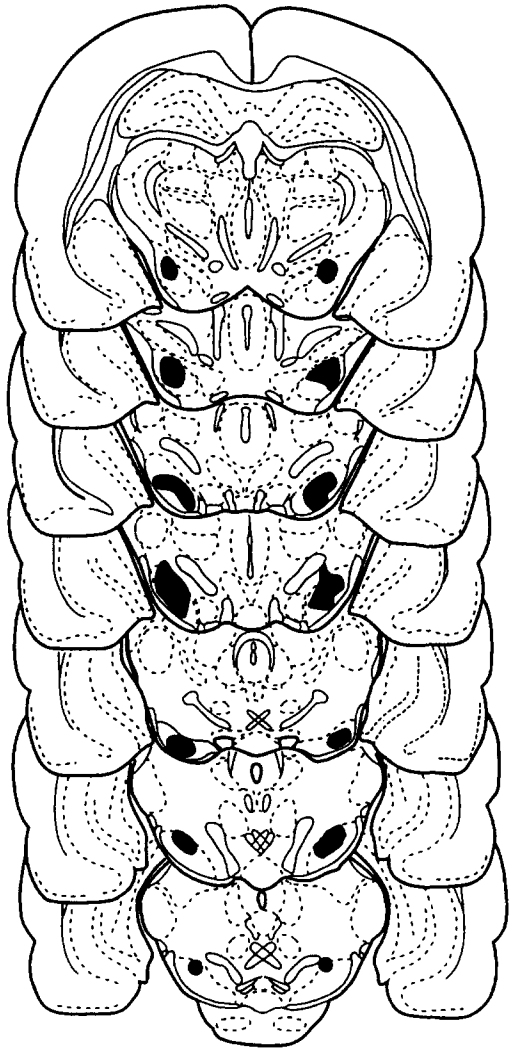

Fig. 1. Histologic verification of the placement of infusion needle tips. Representation of coronal sections through the rat midbrain were taken from the Atlas of Pellegrino et al. (1979). The coronal sections shown display brain structures found between 2.6 (top section) and 4.0 (bottom section) mm caudal to bregma. Only animals with infusion needle tips in the shaded areas were included in the data analyses.

2.6. Histological confirmation of cannula placement

Upon completion of behavioral testing, animals were anesthetized with sodium pentobarbital (100 mg/kg), and killed by decapitation. The brains were removed and placed in 4% formalin until the tissue was fixed (at least 24 h). The brains were then sectioned (40 μm) using a cryostat-microtome (−8°C), and the sections were mounted on gelatin-coated slides and stained with formyl thionin. Histological evaluation of cannula placements and infusion needle tips was made by examining slides on a Nikon profile projector. Histological assessment was made blinded to knowledge of the animal's response to fluoxetine. Only animals with infusion sites within the substantia nigra pars reticulata were included in the statistical analysis (Fig. 1). Rats with infusion sites outside this region served as unique controls.

2.7. Statistical analysis

The latency to the onset of wild running, and to the onset of the convulsion were compared following intranigral microinfusions of 5,7-dihydroxytryptamine, fluoxetine-HCl, or vehicle using a repeated measures analysis of variance with a Scheffé post-hoc test. Seizure severity scores were compared using Mann Whitney U nonparametric analysis. The 5-HT and catecholamine concentrations following 5,7-dihydroxytryptamine treatment were compared to vehicle infused controls using an unpaired Student's t-test. An α level of $P < 0.05$ was maintained throughout the study.

3. Results

3.1. Effect of intranigral 5,7-dihydroxytryptamine on audiogenic seizures in GEPR-3s

GEPR-3s subjected to audiogenic seizure testing 2, 3 and 4 weeks after intranigral infusion of 5,7-dihydroxytryptamine showed no change in seizure behavior when compared to vehicle infused controls. Neither the latency to wild running nor to the convulsion were altered in the three seizure tests following intranigral 5,7-dihydroxytryptamine (Fig. 2A,B). Moreover, intranigral 5,7-dihydroxytryptamine failed to alter the seizure severity scores in GEPR-3s (Fig. 3).

Measurement of regional 5-HT content following intranigral infusions of 5,7-dihydroxytryptamine in GEPR-3s,

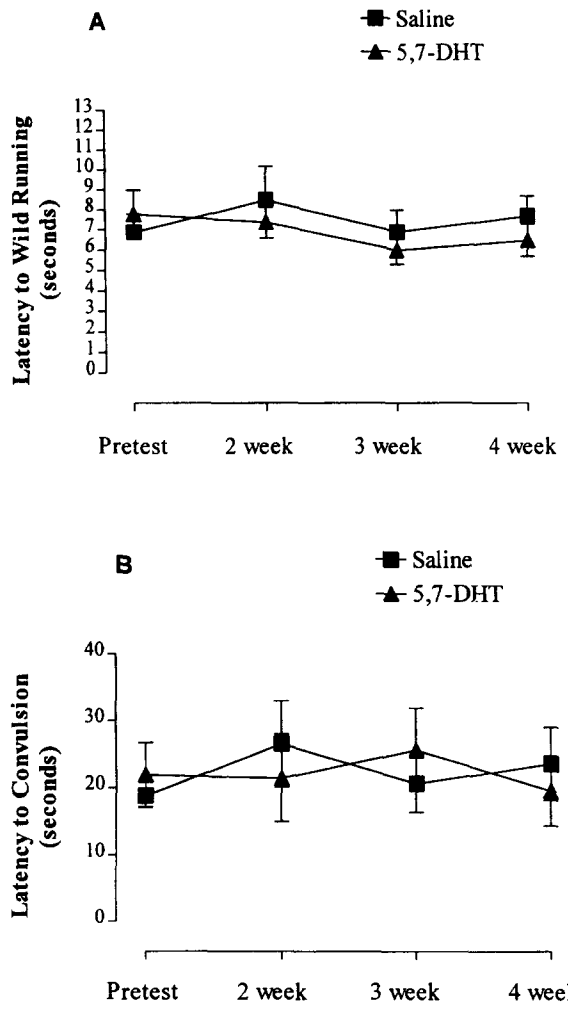

Fig. 2. The effect of intranigral injections of 5,7-dihydroxytryptamine on: (A) the latency to wild running, and (B) the latency to the convulsion in male GEPR-3s during audiogenic seizures. Rats received bilateral injections of either 5,7-dihydroxytryptamine (4 μg/0.25 μl/side) or vehicle (saline-ascorbate) into the substantia nigra pars reticulata. Injections were made into the substantia nigra 2 h following pretreatment with protriptyline (20 mg/kg, i.p.). No significant differences were observed using a one-way analysis of variance with a Scheffé post hoc test between the control and treated groups (represents the average latency ± S.E.M.). Rats per group: control group = 10 and 5,7-dihydroxytryptamine group = 10.

revealed a marked depletion of substantia nigra 5-HT (71% depletion compared to controls) (Table 1). Although less marked, 5-HT was also reduced in the hypothalamus

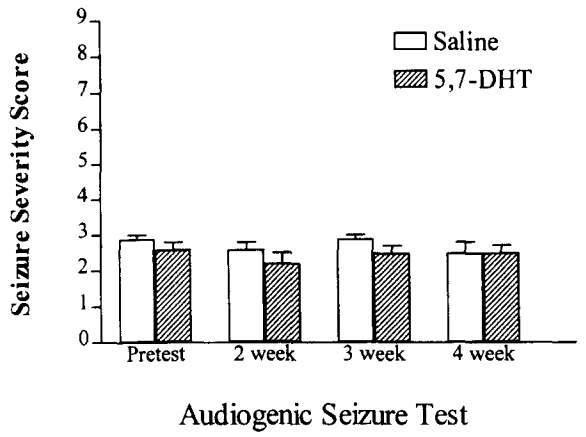

Fig. 3. The effect of intranigral injections of 5,7-dihydroxytryptamine on audiogenic seizure severity in male GEPR-3s. Rats received bilateral injections of either 5,7-dihydroxytryptamine (4 μg/0.25 μl/side) or vehicle (saline-ascorbate) into the substantia nigra pars reticulata. Injections were made into the substantia nigra 2 h following pretreatment with protriptyline (20 mg/kg, i.p.). No significant differences were observed using Mann Whitney U nonparametric analysis between the control and treated groups (represents the average seizure severity score ± S.E.M.). Rats per group: control group = 10 and 5,7-dihydroxytryptamine group = 11.

(43%) and in the midbrain area dorsal to the substantia nigra (37%) of 5,7-dihydroxytryptamine treated GEPR-3s. Protriptyline pretreatment was effective in protecting catecholamine neurons from the neurotoxic effects of 5,7-dihydroxytryptamine, since the concentration of norepinephrine in the midbrain dorsal to the substantia nigra or dopamine in the striatum was not altered in GEPR-3s receiving 5,7-dihydroxytryptamine microinjections (Table 1).

3.2. Effect of intranigral fluoxetine on audiogenic seizures in GEPR-9s

Ten days following implantation of substantia nigra guide cannulas in GEPR-9s, the rats were subjected to audiogenic seizure testing without drug treatment. Only GEPRs displaying a seizure severity score of 9 were allowed to remain in the study. As can be seen in Fig. 4, intranigral microinfusion of fluoxetine, at doses of 3.5, 7.2, or 14.1 nmol/0.5 μl/side, failed to alter audiogenic seizure severity in GEPR-9s 15 min after the infusion. In addition, this treatment failed to influence either the la-

Table 1
Regional depletion of brain monamines in GEPR-3s following intranigral infusions of 5,7-DHT

Region	5-HT (ng/g)		NE (ng/g)		DA (ng/g)	
	Saline	5,7-DHT	Saline	5,7-DHT	Saline	5,7-DHT
Striatum					9301.9 ± 688	8675.5 ± 259
Hypothalamus	1104.4 ± 78	619.5 ± 44 [a]				
Midbrain	859.2 ± 26	542.4 ± 31 [a]	533.2 ± 22	512.1 ± 10		
Substantia nigra	840.5 ± 37	254.7 ± 17 [a]				
Pons-medulla	894.1 ± 79	759.2 ± 32				

5-Hydroxytryptamine, 5-HT; norepinephrine, NE; dopamine, DA; [a] $P < 0.0001$ using an unpaired Student's t-test.

tency to wild running or the latency to the convulsion (Fig. 5A,B).

In view of the widespread innate deficit in the number of 5-HT terminals in the GEPR-9 brain, and the fact that the concentration of 5-HT is innately reduced in the GEPR-9 substantia nigra (Dailey et al., 1992b; Statnick et al., in preparation), it seemed possible that fluoxetine alone was incapable of elevating synaptic 5-HT concentration to the required anticonvulsant level. In order to provide a further increase in the synaptic concentration of 5-HT in the substantia nigra, intranigral fluoxetine was administered to rats following pretreatment with a sub-anticonvulsant dose of D,L-5-hydroxytryptophan (D,L-5-HTP). The D,L-5-HTP (75 mg/kg, i.p.) was administered 30 min prior to seizure testing to acutely increase brain 5-HT levels, and intranigral fluoxetine was given 15 min later (i.e., 15 min before the seizure test according to the procedure for animals receiving fluoxetine microinfusions alone). No change in seizure behavior was observed in GEPR-9s receiving saline in combination with 5-HTP at this dose. As can be seen in Fig. 4, intranigral microinfusions of fluoxetine (at 3.5, 7.2, or 14.1 nmol/0.5 μl/side) in combination with the 5-HTP pretreatment also failed to alter seizure severity in GEPR-9s. Moreover, neither the latency to wild running nor the latency to the convulsion were affected by these treatments (Fig. 5A,B). Although a slight, but nonsignificant, difference between groups was seen on pretest (despite random selection) and after saline

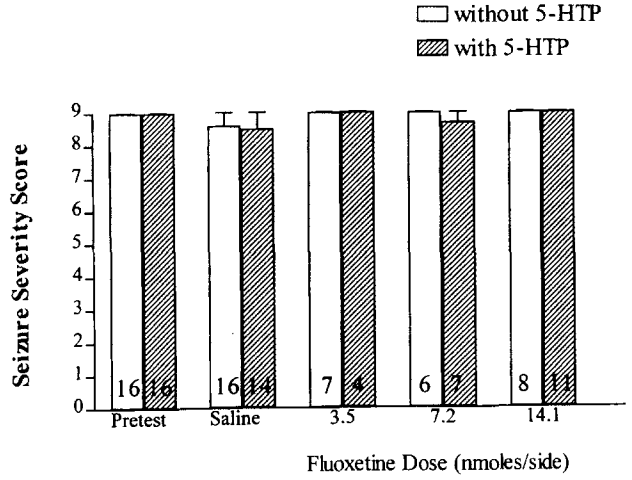

Fig. 4. The effect of intranigral infusions of fluoxetine and fluoxetine in combination with D,L-5-hydroxytryptophan (D,L-5-HTP) pretreatment on audiogenic seizure severity in male GEPR-9s. Rats received drug infusions 10 days following implatation of guide cannulas. Audiogenic seizure tests were conducted 15 min after infusion of fluoxetine (3.5, 7.2, or 14.1 nmol/0.5 μl) or saline (0.5 μl) and 30 min after D,L-5-HTP (75 mg/kg, i.p.) in rats receiving the combination treatment. No difference in audiogenic seizure severity was observed using Mann Whitney U non-parametric analysis in rats treated with fluoxetine alone or fluoxetine in combination with D,L-5-HTP pretreatment when compared to saline alone or saline in combination with D,L-5-HTP. The number of rats per group are indicated inside each bar (represents the average seizure severity score ± S.E.M.).

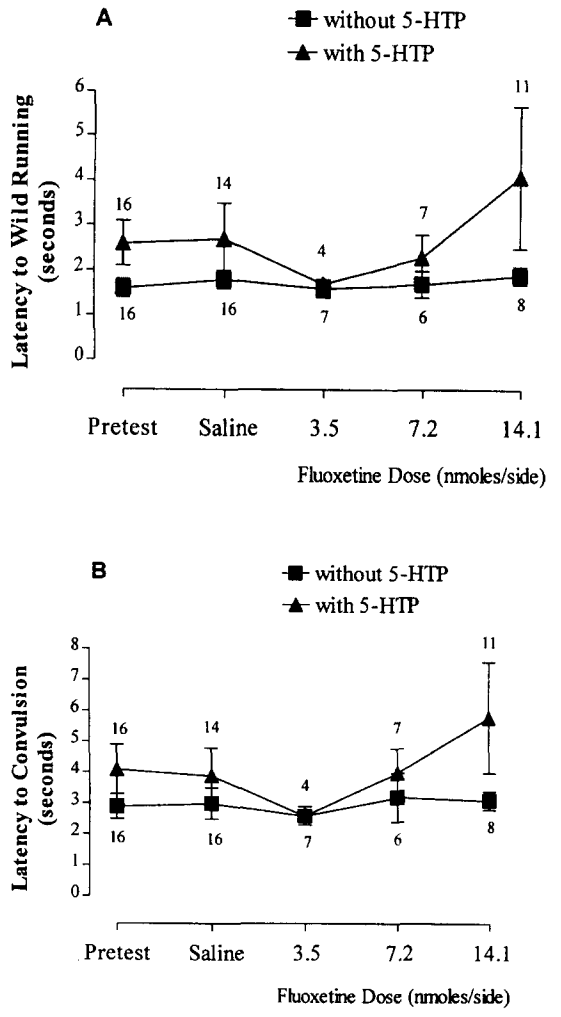

Fig. 5. The effect of intranigral infusions of fluoxetine and fluoxetine in combination with D,L-5-hydroxytryptophan (D,L-5-HTP) pretreatment in male GEPR-9s on: (A) the latency to wild running, and (B) the latency to the convulsion. Rats received drug infusions 10 days following the implantation of guide cannulas. Audiogenic seizure tests were conducted 15 min after infusions of fluoxetine (3.5, 7.2, or 14.1 nmol/0.5 μl) or saline (0.5 μl) and 30 min after D,L-5-HTP (75 mg/kg, i.p.) in rats receiving the combination treatment. No difference in the latency to wild running was observed using one-way analysis of variance with a Scheffé post hoc test in rats treated with fluoxetine alone or fluoxetine in combination with D,L-5-HTP pretreatment when compared to saline alone or saline in combination with D,L-5-HTP. The number of rats per group (represents the average latency ± S.E.M.) are indicated within the figure.

(Fig. 5), the fluoxetine-5-HTP combination failed to alter this relationship.

Of interest in this study was an apparent toxicity of 5-HTP in GEPRs. While the rats appeared normal (except for diarrhea) in the hours immediately following 5-HTP treatment, spontaneous death occurred in a significant percentage (> 30%) of the animals 24–72 h after treatment with 5-HTP. In addition, those animals that survived the treatment with 5-HTP tended to have prolonged latencies to running and to the convulsion when tested 72 h later, although this effect was not statistically significant.

4. Discussion

Having found that chronic widespread damage to the serotonergic neurons leads to an increase in seizure severity in GEPR-3s (Statnick et al., 1992), it was of interest to attempt to identify the brain site(s) which contribute to the enhanced seizure severity following 5-HT depletion. Indeed, finding this site could be tantamount to identifying the site(s) at which 5-HT modulates audiogenic seizures. Based on previous findings showing that intranigral fluoxetine inhibits limbic forebrain seizures (Pasini et al., 1992), and the fact that GEPR-9s display a marked deficit in nigral 5-HT (Statnick et al., in preparation), the substantia nigra appeared to be a potential site at which 5-HT exerts anticonvulsant effects on audiogenic seizures. However, regional 5-HT depletion, unlike widespread central nervous system depletion of 5-HT, in the substantia nigra did not alter audiogenic seizures in GEPR-3s. These findings suggest that the exacerbation of audiogenic seizures produced by i.c.v. 5,7-dihydroxytryptamine in GEPR-3s (Statnick et al., 1992) was not due to a reduction in 5-HT in the substantia nigra alone. Although it is possible that a 71% depletion in substantia nigra 5-HT was insufficient to produce the increase in seizure severity, this seems unlikely since i.c.v. 5,7-dihydroxytryptamine, which enhanced seizure severity, produced only a 52% depletion of brainstem 5-HT (Statnick et al., 1992), and it is doubtful that this treatment depleted the substantia nigra to a greater extent than that observed following focal injections of 5,7-dihydroxytryptamine into the substantia nigra. The depletion of 5-HT was not entirely restricted to the substantia nigra following focal injection of 5,7-dihydroxytryptamine in that the areas immediately adjacent to the substantia nigra (dorsal midbrain and hypothalamus) were partially depleted presumably due to the diffusion of 5,7-dihydroxytryptamine out of the substantia nigra. However, it seems unlikely that this could cause the lack of effect, unless depletion of 5-HT outside the substantia nigra had a seizure attenuating effect; a possibility which seems remote. Thus, based on the present findings one must conclude that the seizure exacerbating effects of i.c.v. 5,7-dihydroxytryptamine (Statnick et al., 1992) and systemically administered parachlorophenylalanine (Jobe et al., 1973a) are not due to a selective loss of 5-HT in the GEPR substantia nigra.

While it does not appear that depletion of 5-HT in the substantia nigra exacerbates audiogenic seizures in the GEPR, there is reason to believe that elevations of neurotransmitters may affect seizures even when depletion does not. Maggio et al. (1991) have shown that selective depletion of nigral GABA failed to potentiate seizures induced by bicuculline or kainic acid. However, increasing GABAergic inhibition in the substantia nigra attenuates seizures induced by maximal electroshock, bicuculline, pentylenetetrazole, flurothyl, and injections of bicuculline into the area tempestas (Iadarola and Gale, 1982; Wurpel et al., 1992; Zhang et al., 1991; Xu et al., 1991; DeSarro et al., 1985; Maggio and Gale, 1989; Xie et al., 1991). This would suggest that tonic GABAergic transmission in the substantia nigra does not normally provide seizure attenuating actions in these seizure models. Moreover, Pasini et al. (1992) have found that microinjections of fluoxetine into the substantia nigra inhibits seizures evoked by injections of bicuculline into the area tempestas, a model of forebrain driven seizures (Browning et al., 1993). This finding suggests that elevating nigral 5-HT neurotransmission affords seizure protection against forebrain driven seizures.

In the present study, audiogenic seizures were not facilitated by depletion of nigral 5-HT in GEPR-3s. However, this finding does not exclude a role for nigral 5-HT in audiogenic seizure regulation, since, as was the case with GABA, it is possible that increments in nigral 5-HT are anticonvulsant, while decrements have no effect. Thus, it seemed reasonable to examine the effect of elevating nigral 5-HT on audiogenic seizures in GEPRs. Previous studies have demonstrated that facilitation of serotonergic transmission by systemic administration of fluoxetine protects rats against audiogenic and limbic motor seizures (Dailey et al., 1992a; Prendiville and Gale, 1993; Yan et al., 1994a, b), and there is evidence that this effect is mediated through 5-HT (Dailey et al., 1992a). Therefore, fluoxetine was employed as the agent to facilitate 5-HT neurotransmission in the substantia nigra. However, focal microinjections of three doses of fluoxetine into the substantia nigra pars reticulata did not affect audiogenic seizures 15 min following drug administration in GEPR-9s. In addition, intranigral injections of fluoxetine in combination with the enhancement of 5-HT synthesis by prior administration of 5-HTP failed to alter audiogenic seizures in GEPR-9s. These findings show that fluoxetine, under the current experimental design, does not attenuate audiogenic seizures through a localized action in the substantia nigra. It seems possible that the lack of an anticonvulsant effect could have resulted from using an inappropriate time (15 min) between drug infusion and audiogenic seizure testing, or an inappropriate dose of fluoxetine. However, Pasini et al. (1992) found that intranigral infusion of identical doses of fluoxetine is effective in protecting rats against limbic motor seizures evoked by injections of bicuculline into the area tempestas 15 min after infusion. Moreover, we have found that intranigral infusion of fluoxetine (14.1 nmol/side) with or without 5-HTP pretreatment failed to produce a change in audiogenic seizures even when tested up to 30 min after infusion of fluoxetine (data not shown). Examination of the infusion used by Pasini et al. (1992) raised the possibility that sites more caudal in the substantia nigra may be important in mediating fluoxetine's anticonvulsant effect, since some of our cannulas were slightly rostral to those of Pasini et al. (1992) within the substantia nigra pars reticulata. However, as indicated above, intranigral injections of fluoxe-

tine (14 nmol/0.5 μl/side) had no effect on audiogenic seizures in GEPR-9s tested 30 min after the infusion (data not shown). This time would easily allow fluoxetine to reach sites within the caudal substantia nigra, since other investigators have found that following microinfusion of 0.5 μl of either [^{14}C]lidocaine in the inferior colliculus or [^{3}H]GABA in the substantia nigra diffusion extended to 1 mm^3 and 0.4 mm^3, respectively (King et al., 1987; Smith et al., 1990). Thus, while it appears that facilitation of serotonergic neurotransmission in the substantia nigra suppresses limbic motor (forebrain driven) seizures (Pasini et al., 1992), it does not appear to suppress audiogenic (brainstem driven) seizures. The anticonvulsant action of intranigral fluoxetine, therefore, may be seizure model-specific.

It seems possible that the seizure model specificity may be due to the innate deficiency in brain 5-HT displayed by GEPRs and not by rats used in other models. Indeed, normal rats have a high concentration of 5-HT in the substantia nigra. However, we have previously shown that GEPR-9s have an innate deficit in steady-state substantia nigra 5-HT levels (Statnick et al., 1991). Although the present study attempted to raise brain 5-HT levels by administration of 5-HTP prior to intranigral injections of fluoxetine, abnormalities in the presynaptic function of 5-HT neurons in GEPRs may have precluded an anticonvulsant action of intranigral applications of fluoxetine, since the action of this drug is dependent on the integrity of presynaptic function of serotonergic neurons. However, this does not seem likely since systemic administration of fluoxetine has been shown to inhibit audiogenic seizures in GEPRs (Dailey et al., 1992a), even though the deficits in a variety of presynaptic 5-HT markers (i.e., 5-HT concentration, synaptosomal 5-HT uptake, and tryptophan hydroxylase activity) occur throughout the GEPR brain (Statnick et al., 1991; Dailey et al., 1992b).

Another explanation that may be invoked to account for the model specificity of nigral 5-HT in modulating seizures is simply that the activity of the substantia nigra does not modulate audiogenic seizures. Indeed, there is much controversy concerning the involvement of the substantia nigra in modulating audiogenic seizures in rats. Recently, unilateral electrolytic lesions of the substantia nigra were found to increase audiogenic seizure susceptibility produced by injections of *N*-methyl-D-aspartate (NMDA) into the inferior colliculus in nonsusceptible Wistar rats, whereas these lesions did not affect audiogenic seizures in a genetically susceptible Wistar strain (Garcia-Cairasco and Sabbatini, 1983, 1991). These findings suggest that the substantia nigra may exert inhibitory tone on audiogenic seizures in normal rats, but not in rats genetically susceptible to audiogenic seizures. Moreover, bilateral injections of muscimol into the substantia nigra (a treatment that has been found to suppress generalized seizures) did not affect audiogenic seizures in genetically susceptible rats or in rats rendered susceptible to audiogenic seizures following ethanol withdrawal (Frye et al., 1983; Depaulis et al., 1990, 1994). The reason for this is not known, but could be due to an abnormal nigral circuitry in the genetically susceptible animals. There are only two reports suggesting that the substantia nigra modulates audiogenic seizures in genetically susceptible rats: one report shows that large bilateral lesions of the substantia nigra protected GEPRs from audiogenic seizures when tested 24 h later (Browning, 1986), and the other shows that microinjection of 2-amino-7-phosphonoheptanoate into the substantia nigra reduced audiogenic seizure severity in GEPR-9s (Millan et al., 1988). Clearly, there are alternative explanations (such as the time of testing after lesions of the substantia nigra) for the latter findings, and more studies are needed to confirm and extend these reports. Without additional information, the most likely explanation for the lack of effect of intranigral fluoxetine on audiogenic seizures is that the substantia nigra plays little or no role in modulating these seizures in GEPRs. Whether this hypothesis is correct will require future studies.

In conclusion, neither inhibition nor enhancement of nigral serotonergic neurotransmission was found to alter audiogenic seizures in GEPRs. Thus, while it appears that 5-HT neurotransmission is an etiologically important determinant of audiogenic seizure severity (Jobe et al., 1973a; Dailey et al., 1992a) in GEPRs, the site(s) of this action does not appear to reside solely within the substantia nigra.

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