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BRIEF COMMUNICATION

Asymmetrical Locomotor Response to Unilateral Cortical Injections of DSP-4

KENNETH L. KUBOS,[1] TIMOTHY H. MORAN, KENNETH M. SAAD AND ROBERT G. ROBINSON

Department of Psychiatry and Behavioral Sciences, Department of Neuroscience Johns Hopkins University School of Medicine, Baltimore, MD 21205

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KUBOS, K. L., T. H. MORAN, K. M. SAAD AND R. G. ROBINSON. *Asymmetrical locomotor response to unilateral cortical injections of DSP-4.* PHARMACOL BIOCHEM BEHAV **21**(1) 163–167, 1984.—The role of norepinephrine depletion in the lateralized production of spontaneous hyperactivity was assessed by unilateral fronto-cortical injections of either 10 or 20 micrograms of the noradrenergic neurotoxin, DSP-4 (N-2-chloroethyl-N-ethyl-bromobenzylamine hydrochloride). Ten μg of DSP-4 produced significant hyperkinesis only when injected into the right hemisphere. A 20 μg dose produced hyperactivity when injected into either hemisphere. DSP-4 injections resulted in significant NE and ipsilateral and contralateral 5HT depletions in the frontal cortex. The 20 μg right hemispheric injection significantly increased both DOPAC and DA levels in the contralateral caudate in a manner unrelated to behavior. These findings, in conjunction with results from previous neurotoxin studies, support a hypothesis of greater relative sensitivity to injury of right hemispheric NE terminal fields compared with injury to left hemispheric fields as demonstrated by spontaneous hyperactivity.

Lateralization Cortical DSP-4 Hyperactivity Norepinephrine 5-Hydroxytryptamine

VARIOUS factors which influence the asymmetrical expression of behavior in the rat have been identified. These include endogenous biogenic amine concentrations [2], handling and environmental enrichment [1] and sex [17]. In recent work [5, 8, 10, 12–16], we have attempted to systematically establish the neurochemical and neuroanatomical substrates of an asymmetrical behavioral response to unilateral cortical injury which we have identified in the rat. While both left and right middle cerebral artery occlusions or focal suction ablations of the frontal cortex produce similar sized lesions, only right hemisphere lesions lead to increased spontaneous activity. This asymmetric behavioral response to ischemic [13] or suction [10] lesions is accompanied by similarly asymmetrical changes in norepinephrine (NE) and dopamine (DA) concentrations.

Two types of chemical lesion experiments have been carried out to date. Cortical injections of kainic acid as a method of producing lesions of cortically resident cell bodies, produced a significantly greater increase in spontaneous activity following right hemispheric injection compared with similar lesions of the homologous left hemisphere [6]. The neurotoxin 6-hydroxydopamine (6-OHDA), which lesions catecholamine neurons, produced hyperactivity when injected at low doses in the right but not the left cerebral cortex [14]. An inherent problem with 6-OHDA, however, is that it destroys both noradrenergic and dopaminergic fibers. While the selectivity of 6-OHDA for dopaminergic neurons can be increased by protective pretreatment with the noradrenergic reuptake inhibitor, desmethylimipramine, it is not yet possible to increase 6-OHDA's selectivity for NE fibers by any similar method. We have sought a technique for producing specific NE lesions. The neurotoxin DSP-4 (N-2-chloroethyl-N-ethyl-2-bromobenzylamine hydrochloride) [4,18] possesses a high selectivity for noradrenergic neurons. In order to establish the role of cortical noradrenergic neurons in the production of lateralized spontaneous hyperactivity we employed localized DSP-4 injections in a paradigm previously used to investigate the lateralization effects of 6-OHDA [14].

METHOD

Male Sprague-Dawley rats (weighing approximately 300 g) were housed individually in cages for 3 weeks prior to operation with food and water freely available in a regular 12-hour light, 12-hour dark environment. The cages consisted of a stationary compartment and a running wheel [11] with free access to either compartment. The running wheel

[1]Requests for reprints should be addressed to Dr. Kenneth L. Kubos, Meyer 4-119, Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, 600 N. Wolfe St., Baltimore, MD 21205.

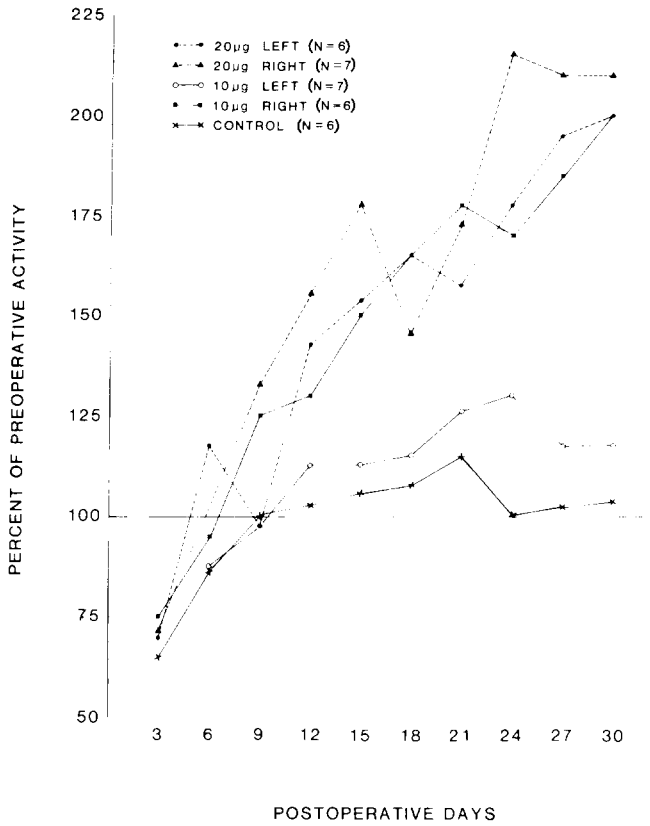

FIG. 1. Effects of DSP-4 dose and hemisphere injected on running wheel activity during the 30 day postoperative period as a percent of preoperative baseline levels established during the week preceding surgery.

could move freely in either direction, and was connected to a cyclometer which was read at 24-hour intervals.

Under Chloropent (3 cc/kg) (Fort Dodge Laboratories, Fort Dodge IA) anesthesia, rats were placed in a stereotaxic apparatus and a frontoparietal craniotomy was performed as described in previous publication [14]. A blunt 29 ga cannula was angled using an electrode carrier such that it entered the brain perpendicular to the surface. Injections were made in the frontal cortex 2.0 mm dorsal to horizontal plane zero and approximately 9.0 mm anterior to ear bar zero [14]. The cannula was lowered 1.5 mm below the cortical surface and withdrawn to 1 mm. One μl injections were made into the cerebral cortex by means of an automated syringe with a delivery rate of 0.33 μl/min. Following drug delivery, the needle was allowed to remain in the brain for an additional 3 min. DSP-4 (N-2-chloroethyl-N- ethyl-2-bromobenzylamine hydrochloride) (supplied by Astra Laboratories) was dissolved in sterile lacted Ringer's solution at a concentration of either 10 or 20 μg/μl. Control injections consisted of vehicle alone, and were delivered identically to drug injections.

Following surgery, animals were returned to their cages and daily postoperative activity was compared with preoperative baseline data, established during the week immediately preceding surgery. Because there were no differences between left and right hemisphere vehicle-injected animals, their data were combined.

At 30 days postoperatively, animals were sacrificed, their brains quickly removed and dissected utilizing a brain slicing apparatus [3] (Research Instruments Inc, San Diego, CA) on a 0°C coldplate as described elsewhere [8]. The areas assayed were taken both ipsilateral and contralateral to the injection site and included frontal cerebral cortex, the head of the caudate, posterior cortex, the substantia nigra, and the locus coeruleus.

After dissection, samples were immediately frozen on dry ice and then transferred to a −80°C low temperature cabinet until analysis by high pressure liquid chromatography with electrochemical detection employing a vitreous carbon electrode operated at +0.70 V referenced to Ag/AgCl as described in previous publication [8].

Data were analyzed by analyses of variance and appropriate planned comparisons.

RESULTS

Behavioral

Sensitivity of spontaneous locomotion to unilateral injections of DSP-4 were distinctly lateralized (Fig. 1).

Following 10 μg injections of DSP-4 into the right hemisphere, rats doubled their preoperative baseline activity by day 27 and daily running wheel revolutions were still ascending by the conclusion of the experiment. Repeated measures analysis of variance revealed that animals receiving injections into the right hemisphere were significantly more active than animals receiving either 10 μg of DSP-4 in the homologous left hemisphere or Ringers control injections $F(2,33)=9.78$, $p<0.01$. The activity of rats receiving 10 μg injections of DSP-4 into the left hemisphere was not found to be significantly different from controls throughout the postoperative period, $F(1,10)=0.45$, $p>0.25$.

Rats receiving unilateral intracortical injections of 20 μg DSP-4 exhibited profound hyperkinesis, $F(2,33)=4.40$, $p<0.05$, irrespective of the injection site, $F(1,11)=0.11$, $p>0.25$. Following surgery, these animals recovered to preoperative activity levels sooner than either 10 μg or control injected subjects and reached 200–210% of their preoperative rates within the 30 day postoperative observation period (Fig. 1). Hyperkinesis exhibited by both left and right hemisphere 20 μg injected and the right hemisphere injected 10 μg groups were similar to each other, $F(1,11)=0.11$, $p>0.25$, and significantly different from both the 10 μg left injected and control groups, $F(1,22)=6.70$, $p<0.05$.

Biochemical

Biogenic amine levels, expressed as a percentage of control values, are presented in Table 1 with significant differences from control values indicated.

Frontal cortex norepinephrine. Analysis of biochemical data revealed significant main effects of dose, $F(2,87)=24.06$, $p<0.001$, lesion side, $F(1,87)=5.95$, $p<0.05$, and side of measurement (ipsilateral-contralateral, $F(1,87)=17.85$, $p<0.001$, with ipsilateral concentrations demonstrating greater depletions than contralateral levels.

Frontal cortex 5-hydroxytryptamine. A significant effect of dosage was demonstrated, $F(2,81)=14.84$, $p<0.001$. Both left hemispheric doses depleted 5HT ipsilaterally and the 20 μg dose caused a contralateral depletion. Ipsilateral, $F(2,22)=4.34$, $p<0.05$, and contralateral, $F(2,22)=4.01$, $p<0.05$, 5HT levels were also decreased by the administration of 20 μg into the right frontal cortex.

TABLE 1

REGIONAL BRAIN AMINE CONTENT 30 DAYS POST DSP-4 (% OF CONTROL ±SEM)

Brain Region (Control pg/mg) ±SE Percent ±SE	Left Hemispheric Dose 10 μg (N=7)	Left Hemispheric Dose 20 μg (N=8)	Right Hemispheric Dose 10 μg (N=7)	Right Hemispheric Dose 20 μg (N=8)
Frontal Cortex				
NE 426 ± 13.3				
100 ± 3.1				
Ipsilateral	68.6 ± 3.2‡	67.4 ± 2.6‡	75.5 ± 5.3†	73.1 ± 4.1†
Contralateral	87.1 ± 6.0*	83.4 ± 3.8†	91.4 ± 4.9	94.0 ± 4.9
5HT 394 ± 22				
100 ± 5.6				
Ipsilateral	69.0 ± 6.4†	67.6 ± 10.3†	89.3 ± 5.2	75.9 ± 10.5*
Contralateral	86.3 ± 15.8	63.1 ± 8.5†	87.3 ± 4.1	76.3 ± 6.9*
Posterior Cortex				
NE 302 ± 9.4				
100 ± 2.9				
Ipsilateral	83.5 ± 2.7†	76.2 ± 2.9‡	92.2 ± 7.5	85.7 ± 2.5*
Contralateral	93.5 ± 1.8	95.0 ± 4.2	88.2 ± 7.7*	90.4 ± 4.5
5HT 264 ± 17.7				
100 ± 3.1				
Ipsilateral	109.5 ± 6.5	76.3 ± 4.6*	95.5 ± 5.5	78.1 ± 4.1*
Contralateral	108.7 ± 6.8	76.0 ± 5.5†	100.7 ± 7.0	85.0 ± 4.2
Locus Coeruleus				
NE 922 ± 66				
100 ± 7.2				
Ipsilateral	84.6 ± 8.5	79.7 ± 9.4*	80.0 ± 5.3	90.7 ± 7.5
Contralateral	73.4 ± 5.3*	86.4 ± 7.5	84.5 ± 6.8	77.4 ± 7.6*
Substantia Nigra				
DA 446 ± 26.6				
100 ± 6.0				
Ipsilateral	81.0 ± 8.4	101 ± 12.3	100 ± 12.1	87.9 ± 10.7
Contralateral	100 ± 12.9	108 ± 11.4	108 ± 7.0	91.2 ± 5.8
Caudate				
DA 8837 ± 415				
100 ± 4.6				
Ipsilateral	90.7 ± 4.4	109 ± 5.8	86.8 ± 5.4	96.7 ± 7.0
Contralateral	100.1 ± 3.9	118 ± 9.3*	87.3 ± 5.2	121.4 ± 6.6†
DOPAC 1668 ± 89				
100 ± 5.6				
Ipsilateral	98.8 ± 4.5	137.7 ± 9.5‡	95.4 ± 4.5	92.4 ± 6.9
Contralateral	102 ± 5.7	123.3 ± 8.6*	98.2 ± 7.4	124 ± 10.1*

*$p<0.05$; †$p<0.01$; ‡$p<0.001$.

Regional amine and metabolite levels as a function of DSP-4 dose and hemisphere injected. Since no interhemispheric differences were noted among controls, tissue values were pooled for the purposes of statistical analysis.

Posterior cortex norepinephrine. There were significant effects of dosage, $F(2,73)=11.64$, $p<0.001$, and measurement side, $F(1,73)=4.51$, $p<0.05$, with ipsilateral concentrations more depleted than contralateral. Furthermore, there was a significant interaction between lesion side and measurement side, $F(1,73)=4.06$, $p<0.05$, evident in a depletion of contralateral NE following the 10 μg right hemispheric DSP-4 dose.

Posterior cortex 5-hydroxytryptamine. A significant effect of dosage was demonstrated, $F(2,82)=17.07$, $p<0.001$, at the 20 μg level.

Locus coeruleus norepinephrine. A significant effect of dosage was demonstrated, $F(2,72)=7.32$, $p<0.01$.

Substantia nigra dopamine. Although no individual values were different from control a significant interaction between dose and measurement side was found, $F(2,73)=4.13$, $p<0.05$.

Caudate. There was a significant effect of dosage evidenced by an elevation of contralateral dopamine concentrations at the 20 μg level, $F(2,80)=13.63$, $p<0.001$. There was also a significant dosage effect upon caudate DOPAC at the 20 mg dose, $F(2,80)=11.61$, $p<0.001$. Furthermore, there

was a main effect on lesion side, $F(1,80)=5.73$, $p<0.05$, with the left being more elevated than the right. A significant interaction, $F(2,80)=3.79$, $p<0.05$, was also found between dosage and lesion side.

CONCLUSIONS

These results indicate that intracortical injections of DSP-4 produce profound hyperkinesis in the rat. At the 10 μg dose only right lesion rats exhibited hyperactivity while left lesion animals were no more active than controls. This lateralized effect of DSP-4 upon spontaneous activity is consistent with our previous findings using neurotoxic [6,14] or mechanical lesions [7, 8, 10]. However, the asymmetrical response was lost at the high, 20 μg dose. That is, under the 20 μg DSP-4 condition, left and right hemisphere treated subjects were significantly more active than controls but not significantly different from each other. The finding, that high doses of neurotoxin injected into the left hemisphere can produce spontaneous hyperactivity is consistent with previous findings using high doses of 6-OHDA or unilateral kainic acid injections [6,14].

Frontal cortex norepinephrine was significantly depleted in the vicinity of the DSP-4 injection site. The observed NE depletions, however, failed to demonstrate a clear relationship either between neurotoxin doses or behavioral activation. There are several possible explanations for the lack of dose related effect of DSP-4 on NE concentrations. Since our injection system delivered a constant volume regardless of dose, toxin spread was presumably confined to a relatively limited, constant area. Thus, we may have reached a ceiling effect with the lower dosage destroying all NE terminals within this limited area. Another possible explanation is that animals were sacrificed 30 days following the lesion and some recovery may have occurred following the higher dosage.

Norepinephrine levels in the locus coeruleus were also reduced by intracerebral DSP-4. These depletions which occurred contralateral to 10 μg left and 20 μg right hemispheric injections (Table 1) reached significance by the analysis of variance. This finding may suggest that intracerebral DSP-4 may extend noradrenergic system damage beyond terminals to perikarya.

DSP-4 lesions in either hemisphere produced significant bilateral serotonin depletions in addition to the expected depletions of NE. Thus, one might speculate that 5HT depletion played a role in the production of hyperactivity. However, several findings suggest that 5HT depletion alone cannot account for the observed lateralized hyperactivity. First, the lower, 10 μg dose, which was effective in eliciting hyperactivity following right frontal cortical injection, failed to produce significant depletions of either ipsilateral or contralateral fronto-cortical 5HT. Secondly, we have recently shown that focal injection of the serotonergic neurotoxin 5,7-dihydroxytryptamine into the frontal cortex of desmethylimipramine pretreated rats failed to alter spontaneous running wheel activity (Black and Robinson, in preparation).

Dopamine and DOPAC concentrations in the caudate were increased by the 20 μg DSP-4 injections (Table 1). However an explanation of the behavioral findings based upon dopaminergic tissue levels alone seems an inadequate explanation for the hyperactivity since the 10 μg right hemispheric DSP-4 dose again did not produce any significant alteration of caudate DA or DOPAC concentrations although it did produce lateralized hyperactivity.

While the hyperactivity inducing effects of 6-OHDA from our previous study [14] could have been interpreted to have resulted from either noradrenergic or dopaminergic damage, the current lack of dopaminergic depletions suggests that the hyperactivity associated with right hemisphere lesions appears to result from the depletion of NE at the cortical level. Thus the present study supports the hypothesis that noradrenergic neuronal injury is sufficient to produce lateralized hyperactivity.

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