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HIPPOCAMPAL AMINO ACID CONCENTRATIONS AFTER RAPHE AND/OR SEPTAL CELL SUSPENSION GRAFTS IN RATS WITH FIMBRIA–FORNIX LESIONS

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Abstract—Two weeks after infracallosal electrolytic fimbria–fornix lesions, Long–Evans female rats received intrahippocampal suspension grafts of either fetal septal or mesencephalic raphe tissue, or a mixture of both. Ten months after lesion surgery, the concentrations of alanine, aspartate, GABA, glutamate, glutamine, glycine, serine and taurine were determined in a dorsal, a "middle" and a ventral region of the hippocampus. We found neither the lesions nor the grafts to have significantly modified the concentration of these amino acids which, in all groups, presented a regional heterogeneity in their hippocampal distribution. GABA, glutamate and glutamine were highest in the ventral hippocampus, whereas the other amino acids were highest in the dorsal region. Our results (i) show that fimbria–fornix lesions do not result in lasting effects on hippocampal concentrations of the assessed amino acids, (ii) confirm the regional heterogeneity in the distribution of these amino acids in the hippocampus and (iii) demonstrate that cell suspension grafts of fetal septal or mesencephalic raphe tissue, as well as grafts of a mixture of both of these tissues, do not exert a non-specific effect on either of the amino acid concentrations measured.

These data complete those of the preceeding paper [Kiss *et al.* (1990) *Neuroscience* **36**, 61–72] concerning the effects of the same grafts on hippocampal cholinergic, serotonergic and noradrenergic markers, as well as on several behavioural variables.

In a series of recent studies, we have investigated the neurochemical effects of intrahippocampal cell suspension grafts which were performed as either single grafts rich in cholinergic or in serotonergic neurons, or as co-grafts of both these neurochemical categories of neurons. These grafts, which were prepared from either the basal forebrain (rich in cholinergic neurons) or the mesencephalic raphe (rich in serotonergic neurons) of the fetal brain, were implanted into the dorsal hippocampus of rats which sustained aspirative lesions of the fimbria, the fornix and all overlying structures.[4,5] Whereas the lesions dramatically decreased the cholinergic and the serotonergic markers in the hippocampus, septal grafts attenuated the cholinergic deficit, raphe grafts attenuated the serotonergic one and co-grafts did so for both deficits. We also found that the grafts that exerted the cholinergic effects produced an attenuation of the lesion-induced increase of hippocampal noradrenaline concentration, a phenomenon which is interpreted as resulting from sympathetic sprouting.[5,6] More recently, we confirmed that such beneficial effects on neurochemical markers of the hippocampus could also be obtained in rats given more partial and more selective damage to the septohippocampal pathways.[12] In this study,[12] we found that co-grafts containing cholinergic and serotonergic neurons were able to attenuate some of the cognitive deficits induced by fimbria–fornix lesions, an effect which was not observed in the rats bearing single grafts.

However, one question that remained open in our last series of experiments[12] was whether the lesions or even the grafts that we used were able to affect neurochemical markers other than only the cholinergic, the noradrenergic and the serotonergic ones. More precisely, we were interested in whether the fimbria–fornix lesions or the intrahippocampal grafts modified hippocampal amino acid concentrations. The reasons to address such a question are multiple.

First, the fimbria–fornix pathways carry GABAergic fibres which originate in the septal region and innervate the hippocampus.[8,13]

Second, grafts of septal origin have been described to contain parvalbumin-positive neurons,[1,3] a subpopulation of GABAergic neurons of the brain.[21] Also, such grafts were recently found to exhibit a dense glutamate decarboxylase-positive immunoreactivity (Hofferer *et al.*, unpublished observations).

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Abbreviations: EDTA, ethylenediaminetetra-acetate; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-ethanesulphonic acid; HPLC, high performance liquid chromatography.

Third, if fimbria–fornix lesions produce the aforementioned denervations of the hippocampus, they also induce some reactional plasticity phenomena which may involve sprouting and/or synaptical reorganization of fibres spared by the lesion (e.g. mossy fibres, entorhino-hippocampal glutamatergic fibres). Some of these fibres use amino acids as neurotransmitter.[10,16,17]

Finally, one cannot exclude that the latter reactional phenomena might also be influenced by intrahippocampal grafts which, as stated previously,[2] may exert some of their morphological and functional effects by the release of neurotrophic factors.[9,14,15,20]

Thus, the present study, which is complementary to that of Jeltsch *et al.*,[12] determined the hippocampal concentrations of alanine, aspartate, GABA, glutamate, glutamine, glycine, serine and taurine in rats which sustained one of the following surgical treatments: a sham operation, a fimbria–fornix lesion, or such a lesion followed by a cell suspension graft of either basal forebrain tissue, mesencephalic raphe tissue or a mixture of both these preparations. The rats were killed about 10 months after lesion surgery and the amino acid concentrations were measured using high performance liquid chromatography (HPLC).

EXPERIMENTAL PROCEDURES

Subjects

This experiment was carried out in 38 Long–Evans female rats from the experiment reported in detail by Jeltsch *et al.*[12]

Surgery

Lesion and transplant surgeries were performed as decribed elsewhere.[3,9,12] Briefly, cells to be grafted were prepared from the brains of Long–Evans fetuses. Two weeks after lesion surgery, a first subgroup of lesioned rats received bilateral intrahippocampal grafts of a cell suspension prepared from the region including the septal–diagonal band of Broca (Group Septum; $n = 8$). A second subgroup of lesioned rats received grafts of a cell suspension prepared from the region including the mesencephalic raphe (Group Raphe; $n = 8$). In a third subgroup, the lesioned rats received co-grafts of a cell suspension in which septal and raphe tissue had been mixed prior to the dissociation (Group Raphe + Septum; $n = 8$). Sham-operated (Group Sham; $n = 7$) and lesion-only (Group Lesion; $n = 7$) rats served as controls. For all other methodological details, see Jeltsch *et al.*[12]

Neurochemical determinations

Tissue preparation. Both hippocampi of each rat were dissected out, separated into three regions (a dorsal, a "middle" and a ventral one) and prepared as described elsewhere.[12] According to their septotemporal level of origin, the left and the right hippocampal regions were collected together into 3 ml of 0.32 M sucrose (in 2.5 mM HEPES, pH 7.4) where they were homogenized in a Potter/Elvehjem glass/Teflon homogenizer (eight strokes at 500 r.p.m.). From this crude homogenate, a 20 μl sample was used for determination of protein content and a further 200 μl aliquot, which was mixed with 800 μl of methanol, was used for the HPLC measurement of the following amino acids: alanine, aspartate, GABA, glutamate, glutamine, glycine, serine and taurine.

Determination of hippocampal amino acid concentrations. After precolumn derivatization, the GABA concentration was measured by isocratic HPLC separation and fluorimetric detection according to the method of Timmerman *et al.*[23] with minor modifications. Separation was achieved at room temperature (21°C) using a Spherisorb ODS-2 column (150 × 4.6 mm i.d., 3 μm) protected with a Nucleosil 10 C18 guard column (10 × 4.6 mm) (Biochrom, France). The mobile phase consisted of 0.05 mol/l Na_2HPO_4, 0.01 mmol/l Na_2EDTA, 0.6% (v/v) tetrahydrofuran and 40% methanol (pH 5.2). For the preparation of the derivatization reagent, 13 mg *o*-phthaldialdehyde was dissolved in 250 μl methanol and added to 2.25 ml of 200 mM $NaHCO_3$ (pH adjusted to 9.5 with a sodium hydroxide solution) containing 10 μl 2-mercaptoethanol. The reagent was prepared daily, left overnight before use and kept on ice in the dark for a day. Ten microlitres of the derivatization reagent were added to a 10 μl homogenate sample. The resulting sample was mixed for 2 min and immediately injected into the HPLC injection valve. The concentration of the other amino acids was measured by HPLC with fluorimetric detection, as described by Donzanti and Yamamoto.[7]

Protein content. Protein content of each homogenate was assessed according to the method described by Lowry *et al.*[18]

Statistical analysis

Data were analysed using an analysis of variance (ANOVA) with two factors (Group × Hippocampal Region) followed, where appropriate, by 2 × 2 comparisons based on Duncan's multiple range test.[22]

RESULTS

In addition to the determination of hippocampal concentrations of amino acids, we also performed histological verifications of the lesion extent or of graft survival, as well as determinations of the graft-induced effects on cholinergic, noradrenergic and serotonergic markers in the hippocampus (for details, see Ref. 12). Briefly, we found all rats to show substantial lesions of both the fimbria and the dorsal fornix. These lesions dramatically reduced the cholinergic and serotonergic markers in the hippocampus. Grafts containing cholinergic neurons fostered cholinergic but not serotonergic recovery, those rich in serotonergic neurons fostered serotonergic but not cholinergic recovery, while co-grafts fostered recovery from both types of deficits. All these neurochemical effects induced by the grafts were more pronounced in the dorsal and the "middle" regions of the hippocampus than in the ventral region.

All data on hippocampal amino acid concentration are presented in Table 1. Whichever amino acid was considered, ANOVA failed to show a significant overall Group effect, suggesting that neither the lesion nor the grafts produced any significant effect on the amino acid concentrations measured in the present experiment. Concerning this overall Group effect, the lowest $F(4/33)$ was about 0.5 (serine) and the lagest was 2.19 (glutamine). ANOVA also showed a significant Hippocampal Region effect on concentration of all amino acids, [$F(2/66) = 81.2$ (alanine), 45.5 (aspartate), 10.4 (GABA), 12.0 (glutamate), 12.6 (glutamine), 119.3 (glycine), 56.9 (serine), 9.3 (taurine), $P < 0.001$ in all cases]. Whichever amino acid was considered, ANOVA did not show any significant Group × Hippocampal Region interaction. The

two by two comparisons showed that the significant region effects were due to the following differences ($P < 0.05$): (i) GABA, glutamate and glutamine concentrations were highest in the ventral region of the hippocampus, whereas (ii) alanine, aspartate, glycine, serine and taurine concentrations were highest in the dorsal region of the hippocampus. For both alanine and serine concentrations we also found significant differences between all three regions. These concentrations were highest in the dorsal region and lowest in the "middle" one (dorsal vs "middle" or ventral, $P < 0.01$; ventral vs "middle", $P < 0.01$).

DISCUSSION

Altogether, our results show that, about 10 months after lesion surgery, fimbria–fornix lesions do not significantly modify the hippocampal concentrations of the eight amino acids measured. Also, single grafts rich in either cholinergic or serotonergic neurons, or co-grafts rich in both these neurochemical categories of neurons, do not significantly alter these amino acid concentrations.

Most of our results obtained in lesion-only rats are in line with those reported recently by Lahtinen *et al.*,[17] who found aspirative lesions of the fimbria, the fornix and the overlying structures (i.e. cingular bundle, cortex and corpus callosum) to be ineffective on hippocampal concentration of aspartate, glutamate, glutamine, glycine and taurine. However, they reported the GABA concentration to be slightly but significantly reduced (by about 11%) in both the dorsal and ventral hippocampus. This finding is at variance with our present study, since we did not find hippocampal GABA concentration to be altered by the lesions. At least two hypotheses might account for this discrepancy. First, Lahtinen *et al.*[17] used a lesion paradigm which damaged both the infracallosal and the supracallosal components of the septohippocampal pathways, whereas our lesions only altered the infracallosal one. Thus, it is possible that Lahtinen *et al.*[17] destroyed septohippocampal GABAergic axons that have not been damaged by our less extended lesions. Alternatively, one might also consider the time which has elapsed between lesion surgery and death. In our experiment this time was about 10 months, whereas Lahtinen *et al.*[17] made their measurement four months postsurgery. Regarding this difference, it is possible that some time-dependent compensatory phenomena such as, for instance, sprouting of undamaged GABAergic fibres, may have compensated for an initial lesion-induced deficit which was still detectable four months after surgery[17] but not at 10 months (present experiment).

The longitudinal and lateral regional heterogeneities of the hippocampal amino acid concentrations have been described in detail recently by Hörtnagl *et al.*[11] Our present experimental approach did not

Table 1. Average values (S.E.M.) of amino acid concentrations found in the three hippocampal regions of sham-operated rats ($n = 7$), lesion-only rats ($n = 7$) and lesioned rats with raphe ($n = 8$), septal ($n = 8$) or a mixture of raphe and septal ($n = 8$) grafts

Neurochemical marker		Surgical treatment				
		Sham	Lesion	Raphe	Septum	Raphe + septum
Alanine	Dorsal	0.5 (0.1)	0.6 (0.1)	0.4 (0.1)	0.4 (0.1)	0.5 (0.1)
	"Middle"	0.2 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)
	Ventral	0.2 (0.1)	0.3 (0.1)	0.2 (0.1)	0.3 (0.1)	0.3 (0.1)
Aspartate	Dorsal	47.1 (2.1)	51.1 (4.7)	45.2 (2.7)	45.5 (2.0)	53.8 (4.4)
	"Middle"	33.6 (3.1)	33.7 (3.5)	33.3 (4.7)	32.6 (3.2)	33.3 (3.7)
	Ventral	34.6 (1.8)	37.5 (3.2)	35.0 (1.8)	33.9 (2.7)	36.4 (4.1)
GABA	Dorsal	17.2 (1.0)	18.5 (1.9)	15.4 (1.4)	16.2 (0.8)	20.3 (2.1)
	"Middle"	18.9 (1.6)	19.0 (1.0)	19.6 (1.7)	19.9 (1.1)	21.4 (1.1)
	Ventral	21.7 (1.0)	21.0 (0.8)	19.7 (0.9)	19.6 (0.5)	20.4 (1.7)
Glutamate	Dorsal	87.1 (4.3)	87.2 (5.7)	80.5 (5.1)	77.4 (2.6)	92.6 (6.3)
	"Middle"	79.6 (4.7)	79.7 (5.6)	80.2 (5.6)	77.6 (2.9)	87.2 (5.1)
	Ventral	97.1 (4.0)	87.9 (3.5)	95.2 (5.0)	90.8 (4.3)	103.2 (8.3)
Glutamine	Dorsal	101.9 (4.2)	117.3 (8.1)	99.8 (4.9)	97.9 (3.5)	119.0 (9.5)
	"Middle"	95.4 (7.2)	96.1 (6.7)	100.4 (6.9)	94.5 (3.1)	108.3 (5.2)
	Ventral	116.1 (5.2)	108.7 (5.7)	117.0 (6.5)	111.1 (6.2)	130.4 (9.9)
Glycine	Dorsal	31.5 (3.0)	43.9 (5.6)	32.1 (3.4)	31.2 (2.9)	35.6 (4.7)
	"Middle"	15.0 (1.6)	13.7 (1.2)	14.1 (3.3)	13.0 (1.3)	14.1 (1.3)
	Ventral	14.7 (1.3)	16.6 (2.0)	14.9 (1.2)	15.5 (1.5)	15.7 (2.6)
Serine	Dorsal	30.0 (5.2)	46.8 (10.8)	33.2 (7.0)	29.5 (4.2)	32.8 (5.7)
	"Middle"	9.9 (2.5)	9.0 (1.6)	8.6 (0.8)	7.6 (0.7)	9.2 (0.7)
	Ventral	15.6 (1.7)	20.0 (2.8)	16.8 (1.4)	18.6 (2.5)	19.4 (3.9)
Taurine	Dorsal	80.6 (6.4)	80.4 (5.4)	74.1 (5.3)	70.4 (2.8)	87.0 (6.0)
	"Middle"	70.3 (5.7)	71.0 (6.8)	70.7 (6.6)	69.7 (3.7)	81.0 (6.9)
	Ventral	71.0 (4.5)	60.9 (2.3)	67.4 (4.1)	63.3 (2.1)	67.2 (7.2)

The data are given in nmol/mg protein.

Statistics: there was no significant overall group effect on any amino acid concentration but, for each amino acid, there was a significant heterogeneity among the three hippocampal regions (see text for further details).

allow us to address whether there was a lateral heterogeneity in the distribution of the amino acids measured, but our data concerning the longitudinal distribution of these amino acids (comparison between dorsal and ventral hippocampal regions) are in line with those described by Hörtnagl *et al.*[11] These authors[11] have separated the hippocampus into only two regions along its septotemporal axis. In our present experiment, we have dissected the hippocampus into three regions along this axis and, as found with alanine and serine, our results indicate that the dorsal to ventral gradient does not necessarily follow a regular increasing or decreasing trend.

As reported elsewhere,[12] we found that, in rats given fimbria–fornix lesions, intrahippocampal grafts rich in cholinergic neurons fostered cholinergic effects, grafts rich in serotonergic neurons fostered serotonergic effects, while co-grafts combined the respective properties of each single graft. Since in our present experiment we did not only confirm our previous neurochemical results,[4,5,12] but also found that no graft-induced effect could be detected on the concentration of the principal hippocampal amino acids, we assume that the cholinergic and/or serotonergic graft-induced effects may reach an unexpectedly high degree of specificity, depending upon the anatomical origin of the grafted cells.

However, the fact that neither the fimbria–fornix lesion nor the grafts altered the concentration of the hippocampal amino acids measured should not be regarded as indicating that one or the other of these treatments had no effect at all on the hippocampal neurophysiology or neuropharmacology related to these amino acids. Actually, fimbria–fornix lesions may elicit sprouting of mossy fibres or of fibres arising in the entorhinal cortex, and may thereby contribute to modify more functional markers of amino acid neurophysiology (e.g. number and/or sensitivity of presynaptic and postsynaptic receptors, turnover and release of amino acids[10,16]) without altering the hippocampal amino acid concentrations. As an illustration of such a possibility, Marchi and Raiteri[19] have reported that glutamate release by hippocampal synaptosomes could be inhibited by muscarinic activation. Also, Lahtinen *et al.*[16] have shown that L-[^{3}H]glutamate binding in the hippocampus could be altered by a fimbria–fornix transection, whereas such a lesion had no effect on the hippocampal glutamate concentration[17] (see also Ref. 10). In addition, it is also clear that endogenous levels of amino acids such as, for instance, GABA, aspartate or glutamate, can be considered as a poor index of functional alterations consecutive to lesions or transplants. Future experiments should be based on pharmacological or histological markers of hippocampal amino acid functionality after denervation and intrahippocampal transplantation of cerebral tissue.

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