Role of Glucocorticoids in the Cholinergic Degeneration in Rat Hippocampus Induced by Ethylcholine Aziridinium (AF64A)

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Glucocorticoids potentiate hippocampal damage induced by various noxious insults *in vivo* and *in vitro* and are implicated in age-related loss of neurons in the hippocampus of various species. The cholinergic innervation of the hippocampus appears to be especially prone to the endangering effect of glucocorticoids, since corticosterone, like acute stress or ACTH, induces a rapid activation of the cholinergic septohippocampal pathway. We now report the influence of glucocorticoids on the degeneration of this pathway induced by the cholinergic neurotoxin ethylcholine aziridinium (AF64A). The toxic effect of a submaximal dose of AF64A on cholinergic neurons was evaluated in rats during exposure to glucocorticoids or vehicle as well as in adrenalectomized or sham-operated rats. Daily treatment with either corticosterone or dexamethasone, starting 7 d before the bilateral intracerebroventricular injection of AF64A (1 nmol/ventricle), significantly increased the AF64A-induced loss of ChAT activity in the whole hippocampus, whereas bilateral adrenalectomy 7 d prior to AF64A-injection attenuated the effect of AF64A. Short-term exposure to corticosterone starting 24 hr before AF64A was as effective as the 7 d pretreatment. Dexamethasone exacerbated the AF64A-induced cholinergic lesion in the hippocampal subregions CA1, CA3, and dentate gyrus, and adrenalectomy protected all subregions against the action of AF64A. Along the longitudinal axis of the hippocampus a comparable influence was seen in the dorsal and ventral parts. The subregional pattern in the response to glucocorticoid suggests the involvement of mineralocorticoid type I receptors.

In conclusion, glucocorticoids enhance the susceptibility of the septohippocampal cholinergic neurons to AF64A and probably do this by increasing the velocity of the high-affinity choline transport and stimulating the noradrenergic function. The present data suggest a pathophysiological link between glucocorticoids and the age-dependent decline in basal forebrain cholinergic function, with possible implications for the process of cholinergic degeneration occurring in Alzheimer's disease.

[Key words: glucocorticoids, adrenalectomy, hippocampus, ethylcholine aziridinium (AF64A), basal forebrain cholinergic systems, Alzheimer's disease]

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The hippocampus is the primary target tissue for glucocorticoids in the brain. In the rat brain this structure contains the highest number of glucocorticoid-concentrating neurons (McEwen et al., 1968; Sapolsky et al., 1983a). Two types of corticosteroid receptors with differing topographic distribution in rat hippocampus have been described (Reul and deKloet, 1985; Van Eekelen et al., 1988; Herman et al., 1989; Stumpf et al., 1989). The glucocorticoid receptors (type II) that show high-affinity for the synthetic glucocorticoid dexamethasone are mainly restricted to the hippocampal subregions CA1, CA2, and dentate gyrus, whereas the mineralocorticoid receptors (type I), which bind corticosterone as well as the mineralocorticoid aldosterone, are present and more abundant in all subregions of the hippocampal formation, with highest density in CA3.

There appears to be a close link between glucocorticoids and hippocampal vulnerability and damage (Sapolsky, 1987). Glucocorticoids in supraphysiological concentrations not only have been suggested to contribute to age-related loss of neurons in the hippocampus of rats (Sapolsky et al., 1985), guinea pigs (Ausder Muhlen and Ockenfels, 1969), and primates (Sapolsky et al., 1990), but also have been shown to potentiate hippocampal damage induced by various noxious insults *in vivo* and *in vitro*. This phenomenon has been demonstrated in several experimental conditions. Glucocorticoids endanger hippocampal neurons exposed to hypoxia-ischemia (Sapolsky and Pulsinelli, 1985; Koide et al., 1986), while adrenalectomy protects hippocampal pyramidal cells from transient ischemia in rats (Morse and Davis, 1990). Glucocorticoids potentiate, whereas adrenalectomy attenuates, kainic acid-induced seizures and wet dog shakes as well as the extent of hippocampal damage (Sapolsky, 1985a; Lee et al., 1989). Furthermore, glucocorticoids increase the neurotoxicity of the amphetamine analog 3,4-methylenedioxymethamphetamine on the serotonergic system only in the hippocampus and not in the cortex of rats (Johnson et al., 1989). The toxicity of glucocorticoids has been confirmed *in vitro* in primary cultures of dispersed fetal rat hippocampal neurons (Sapolsky et al., 1988).

The cholinergic innervation of the hippocampus appears to be prone especially to the endangering effect of glucocorticoids. Evidence is available that the septohippocampal cholinergic system undergoes rapid activation after acute stress as indicated by increases in both high-affinity choline transport (HChT) and ACh release (Finkelstein et al., 1985; Gilad et al., 1985; Imperato et al., 1989). Increased release of hippocampal ACh also occurred after administration of corticosterone (Imperato et al., 1989). The data imply that hippocampal cholinergic afferents are possibly involved in the brain's control of stress response. Therefore, the question arises of whether this cholin-

ergic system, which strongly responds to stress reactions, might be especially vulnerable during prolonged stress or excess glucocorticoid exposure.

The purpose of the present study was to define the role of glucocorticoids in the vulnerability of the cholinergic septohippocampal pathway to the damaging effect of the cholinergic neurotoxin ethylcholine aziridinium (AF64A). After intracerebroventricular application, AF64A has the potential to destroy preferentially and irreversibly the cholinergic innervation of the rat hippocampus, based on the evidence of immunohistochemical and biochemical studies (Gaál et al., 1986; Hörtnagl et al., 1987a,b; Leventer et al., 1987). We also intended to determine whether glucocorticoid or mineralocorticoid receptors are involved in such a process. To reach this goal, the toxic effect of a submaximal dose of AF64A (Hörtnagl et al., 1987b) on cholinergic neurons was evaluated during exposure to excess of dexamethasone or corticosterone or after adrenalectomy. In addition, the extent of the AF64A-induced cholinergic deficit following either glucocorticoid exposure or adrenalectomy was investigated in various subfields of the hippocampus, namely, CA1, CA3, and dentate gyrus. Furthermore, the effect of glucocorticoids or adrenalectomy on the level of various neurotransmitter markers in the hippocampus of control rats was studied.

Materials and Methods

Animals. Male Sprague–Dawley rats (Forschungsinstitut für Versuchstierzucht, Himberg, Austria) with a body weight of 280–370 gm were used for these experiments. The animals were housed in groups of three per cage, on a 12 hr light, 12 hr dark cycle, and had free access to laboratory chow and tap water.

Materials. ^{14}C -acetyl coenzyme A and L-1- ^{14}C -glutamic acid (specific activities, 50–55 mCi/mmol) were purchased from Amersham Laboratories (UK); acetyl coenzyme A, from Boehringer Mannheim (Germany). Corticosterone, dexamethasone, noradrenaline (NA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) were obtained from Sigma Chemie GmbH (Deisenhofen, Germany). AF64A was prepared from acetylthylcholine mustard according to Fisher et al. (1982). Acetylthylcholine mustard was a gift from Dr. I. Hanin (Loyola University, Chicago).

AF64A-injection. Rats, anesthetized with chloral hydrate (325 mg/kg, i.p.), received stereotaxic infusions (0.5 $\mu\text{l}/\text{min}$) of 1 nmol of AF64A in 3 μl of vehicle into each lateral ventricle. Stereotaxic coordinates were from bregma: posterior 0.8 mm, lateral ± 2.5 mm, ventral (from dura) 3 mm. A small-animal stereotaxic frame (David Kopf Instruments) was used.

Glucocorticoid treatment. For long-term exposure, dexamethasone was administered subcutaneously in 1 ml of sesame oil at a dose of 1.5 mg/kg on days 1 and 2. On days 3–5 the dose was reduced to 1 mg/kg. Thereafter, 1 mg/kg was applied every second day until day 14. Control rats received corresponding injections of sesame oil. For long-term treatment with corticosterone, rats were injected subcutaneously with daily doses of 10 mg of corticosterone in 1 ml of sesame oil for 14 d. In experiments of Sapolsky et al. (1985) this dose of corticosterone suspended in sesame oil produced circulating corticosterone concentrations equivalent to those seen after major stressors for the greater part of the day. Control rats were given daily subcutaneous injections of 1 ml sesame oil. In both experiments AF64A or vehicle was injected on day 8 and rats were killed by decapitation on day 15.

For short-term exposure rats received only three subcutaneous injections of corticosterone (10 mg in 1 ml of sesame oil) 24 hr and 15 min before and 24 hr after AF64A or vehicle, respectively. The control group was given the corresponding subcutaneous injections of sesame oil. Rats were killed 7 d after the stereotaxic infusion of AF64A or vehicle.

Adrenalectomy. Bilateral adrenalectomy or sham operation was performed through dorsal incisions in chloral hydrate anesthesia (325 mg/kg, i.p.) 7 d before the application of AF64A or vehicle. Adrenalectomized animals were supplied with 0.9% saline instead of water. In a group of adrenalectomized rats glucocorticoid supplementation was started 2 d before the injection of AF64A (1.5 mg corticosterone/day

supplied in the drinking fluid). After adrenalectomy or sham operation the rats were single housed.

Dissection of the brains. All groups of rats were killed 7 d after AF64A or vehicle. After decapitation the brains were rapidly removed and immediately chilled in iced water kept at -0.8°C with NaCl. After 10 min of chilling the parietal cortices and hippocampi were removed and frozen on dry ice. In some of the experiments the hippocampi were dissected out and gently extended on a cold plate ($+1^\circ\text{C}$), with the inner surface facing upward. The extended hippocampi were subdivided into three parts: CA1, CA3, and dentate gyrus. As described by Berger et al. (1986) the hippocampi were first cut along the minor fissure into a CA3 part, containing most of the CA3 region, and the dentate gyrus/CA1 part. Separation between CA1 and dentate gyrus was achieved by carefully opening the hippocampal fissure. In some experiments all three subregions were additionally divided into a ventral and dorsal half (for more detail, see Hörtnagl et al., 1991b). The corresponding samples of the left and right hippocampus were combined, weighed, immediately frozen on dry ice, and stored at -80°C until biochemical analysis.

Biochemical analyses. Tissue samples were homogenized by ultrasonication in 20–40 vol of N_2 -saturated deionized water. Immediately after sonication an aliquot of the homogenate (200–300 μl) was added to an equal volume of 0.2 M perchloric acid containing 0.8 mM NaHSO_3 and centrifuged at $25,000 \times g$ at 4°C for 15 min. The supernatant was used for the measurement of NA, 5-HT, 5-HIAA, and the putative neurotransmitter amino acids. The remaining aqueous homogenate was used for the measurement of activities of choline acetyltransferase (ChAT) and glutamate decarboxylase (GAD).

Enzyme activities. ChAT activity was measured according to Fonnum (1969) with minor modifications using substrate concentrations of 8 mM choline chloride and 0.2 mM acetyl coenzyme A (Sperk et al., 1983). GAD was measured in the supernatant, after centrifugation of the aqueous homogenate at $15,000 \times g$ for 10 min, by trapping $^{14}\text{CO}_2$ formed from L-1- ^{14}C -glutamic acid as described by Roberts and Simonson (1963) at substrate concentrations of 15 mM glutamic acid and 0.5 mM pyridoxal phosphate.

Determination of monoamine, 5-HIAA, and GABA levels. NA was measured by high-performance liquid chromatography (HPLC) with electrochemical detection after adsorption to alumina according to Felice et al. (1978) with minor modifications (Sperk et al., 1981). 5-HT and 5-HIAA were determined by HPLC with electrochemical detection as described previously (Sperk, 1982). The levels of GABA were measured by HPLC after precolumn derivatization with the fluorogenic reagent *O*-phthalaldehyde according to the method described by Donzanti and Yamamoto (1988) using an Aminco fluoromonitor (360/450 nm) for the quantification of the amino acid derivative.

Data analyses. All data are presented as means $\pm$ SEM. For statistical analyses the two-factor analysis of variance (ANOVA) with repeated measures was applied when comparing the levels in the various subregions of the hippocampus. For more detailed analyses of eventual differences between the various treatments, a two-tailed Student's *t* test or a Newman–Keuls test with α -adjustment according to Bonferroni was used as posteriori test. When the effects of treatments were compared in the whole hippocampus, the one-way ANOVA followed by the Newman–Keuls test was applied. The significance level assumed was $\alpha = 0.05$. For the data expressed as percentage of vehicle-injected rats, the statistical difference was calculated from the actual levels.

Results

Effects of glucocorticoid exposure or adrenalectomy on body weight

Since glucocorticoids have a considerable impact on metabolism and energy supply and this effect has been linked to their neurotoxicity (Sapolsky, 1985a, 1986), the body weight of the rats was checked during glucocorticoid exposure or after adrenalectomy. Marked changes in body weight were observed. Especially during long-term treatment with dexamethasone or corticosterone, considerable losses in body weight were apparent, reaching up to 80 gm (about 25% of the original body weight) after 2 weeks of exposure. During the short-term exposure to corticosterone, however, the fluctuations in body weight were much smaller (maximal loss, 20 gm). After adrenalectomy the loss in

Table 1. Effect of adrenalectomy on various neuronal markers in hippocampal subregions of the rat

		CA1	CA3	Dentate gyrus
NA (ng/mg tissue)	Sham operation	0.290 ± 0.013	0.431 ± 0.018	0.515 ± 0.024
	Adrenalectomy	0.207 ± 0.014**	0.317 ± 0.014**	0.413 ± 0.019*
5-HT (ng/mg tissue)	Sham operation	0.255 ± 0.012	0.388 ± 0.018	0.212 ± 0.009
	Adrenalectomy	0.241 ± 0.018	0.380 ± 0.030	0.231 ± 0.014
5-HIAA (ng/mg tissue)	Sham operation	0.313 ± 0.011	0.399 ± 0.019	0.345 ± 0.015
	Adrenalectomy	0.452 ± 0.089	0.452 ± 0.035	0.426 ± 0.024*
ChAT (nmol/hr/mg tissue)	Sham operation	6.85 ± 0.19	5.11 ± 0.11	6.48 ± 0.14
	Adrenalectomy	6.76 ± 0.22	4.79 ± 0.16	6.81 ± 0.20
GAD (nmol/hr/mg tissue)	Sham operation	15.53 ± 0.46	19.42 ± 0.43	22.16 ± 0.58
	Adrenalectomy	16.82 ± 0.45	19.57 ± 0.47	23.93 ± 0.38

Sham operation, $n = 12$; adrenalectomy, $n = 7$. *, $p < 0.05$; **, $p < 0.01$; versus sham-operated rats.

body weight as compared to sham operation did not exceed 30 gm.

Influence of excess exogenous glucocorticoids or absence of endogenous glucocorticoids on various neurotransmitter markers

After adrenalectomy a decline in the level of NA was found in the vehicle-injected group of rats, in all three subregions of the hippocampus (Table 1). With regard to the serotonergic system, a slight increase in 5-HIAA, which proved to be significant in the dentate gyrus, was associated with unchanged 5-HT levels. The activities of ChAT and GAD were not affected by the lack of endogenous glucocorticoids produced by adrenalectomy. However, long-term exposure to excess of dexamethasone induced a significant increase in GAD activity in CA1 and dentate gyrus. This increase did not occur in CA3 or in the parietal cortex. The increase in GAD activity was not associated with a change in GABA levels (Table 2). During long-term exposure to corticosterone, however, GAD activity remained unaltered. Monoaminergic or cholinergic parameters were not influenced by dexamethasone and corticosterone (data not shown).

Neurotoxicity of AF64A in the various hippocampal subfields

The effect of AF64A on the activities of ChAT and GAD and on the monoaminergic systems is summarized in Table 3. In the control (vehicle-injected) rats subregional differences in neurotransmitter markers were apparent. Lowest ChAT activity but highest 5-HT and 5-HIAA levels were measured in CA3 ($p < 0.01$); the dentate gyrus contained the highest levels of NA and

GAD activity ($p < 0.01$). In AF64A-treated rats (1 nmol/ventricle) CA3 was the hippocampal area with the highest reduction of ChAT activity ($57.1 \pm 4.5\%$ as compared to $46.7 \pm 4.0\%$ and $49.4 \pm 4.2\%$ in CA1 and dentate gyrus, respectively). Besides the cholinergic deficit, changes in serotonergic function were observed. The decrease in 5-HT, which was most pronounced in CA3 ($p < 0.01$), was not associated with a significant change in the levels of 5-HIAA. The levels of NA were only slightly reduced by maximally $17.6 \pm 4.0\%$ ($p < 0.01$) in CA3. The activity of GAD remained unchanged in all hippocampal subregions.

Effect of glucocorticoids on the AF64A-induced cholinergic lesion

Both dexamethasone (up to 1.5 mg/kg/d, s.c.) and corticosterone (10 mg/d, s.c.) exaggerated the cholinergic deficit in the rat hippocampus following bilateral intracerebroventricular injection of a submaximal dose of AF64A. The potentiation of the toxicity of AF64A after treatment with the glucocorticoid receptor ligand dexamethasone was most pronounced in CA3 and dentate gyrus (Fig. 1). The influence of the mineralocorticoid receptor ligand corticosterone on the whole hippocampus is summarized in Table 4. As can be seen from the table, corticosterone already potentiated the loss of ChAT activity when given around the time point of AF64A injection (24 hr and 15 min before and 24 hr after AF64A). A prolonged exposure to corticosterone (daily injections starting 7 d before AF64A and continuing for 7 d after AF64A) did not further increase this exacerbation of the AF64A-induced damage. In the parietal cortex the slight,

Table 2. Effect of dexamethasone on the activity of GAD and GABA levels in hippocampal subregions and parietal cortex of the rat

	Hippocampus			Parietal cortex
	CA1	CA3	Dentate gyrus	
GAD (nmol/hr/mg tissue)				
Sesame oil/vehicle	19.61 ± 0.55	19.89 ± 0.52	23.41 ± 0.46	22.05 ± 0.62
Dexamethasone/vehicle	22.52 ± 0.33**	21.64 ± 0.73	26.29 ± 1.03*	23.26 ± 0.61
GABA (ng/mg tissue)				
Sesame oil/vehicle	211 ± 8	392 ± 22	471 ± 21	n.e.
Dexamethasone/vehicle	207 ± 4	376 ± 21	496 ± 9	n.e.

Sesame oil/vehicle, $n = 6$; dexamethasone/vehicle, $n = 5$. n.e., not estimated.

*, $p < 0.05$; **, $p < 0.01$; versus vehicle-treated rats.

Table 3. Effect of AF64A (1 nmol/ventricle), 7 d after intracerebroventricular injection, on ChAT and GAD activities and monoaminergic parameters in various subregions of the rat hippocampus

Neuronal marker	Treatment	CA1	CA3	Dentate gyrus
ChAT activity (nmol/hr/mg tissue)	Vehicle	6.58 ± 0.17	5.32 ± 0.12	6.64 ± 0.14
	AF64A	3.51 ± 0.26***	2.28 ± 0.24***	3.36 ± 0.28***
5-HT (ng/mg tissue)	Vehicle	0.271 ± 0.012	0.398 ± 0.016	0.235 ± 0.012
	AF64A	0.221 ± 0.013*	0.259 ± 0.017***	0.172 ± 0.010**
5-HIAA (ng/mg tissue)	Vehicle	0.344 ± 0.014	0.423 ± 0.016	0.366 ± 0.014
	AF64A	0.326 ± 0.014	0.393 ± 0.019	0.321 ± 0.009
NA (ng/mg tissue)	Vehicle	0.293 ± 0.008	0.426 ± 0.012	0.495 ± 0.019
	AF64A	0.253 ± 0.007**	0.351 ± 0.017**	0.423 ± 0.015*
GAD activity (nmol/hr/mg tissue)	Vehicle	16.89 ± 0.58	19.58 ± 0.33	22.57 ± 0.43
	AF64A	17.14 ± 0.44	18.32 ± 0.41	22.94 ± 0.38

Vehicle-treated rats, $n = 18$; AF64A-injected rats, $n = 16$. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; versus vehicle-treated rats.

yet insignificant, reduction in ChAT activity in response to AF64A (3.02 ± 0.12 vs. 3.35 ± 0.12 nmol/hr/mg tissue in vehicle-injected rats) was not potentiated by dexamethasone.

Influence of adrenalectomy on the susceptibility of the hippocampus to AF64A

Adrenalectomy, 7 d prior to AF64A administration, considerably protected all subregions of the hippocampus against the toxicity of AF64A. In CA3 the AF64A-induced loss of ChAT activity was no longer significant after adrenalectomy (Fig. 2). In order to determine the possible involvement of adrenal glucocorticoids as a contributory factor in the AF64A toxicity, adrenalectomized rats were subjected to an oral replacement therapy with corticosterone. This supplementation almost com-

pletely restored the capacity of AF64A to damage the cholinergic system. As can be seen in Figure 2, in adrenalectomized rats treated with daily doses of 1.5 mg corticosterone, the AF64A-induced reduction in ChAT activity did not differ significantly from that in sham-operated rats. This was the case despite the fact that the replacement was started only 2 d before AF64A administration and thus the plasma corticosterone levels may not have reached steady state values.

Role of glucocorticoids in the ventral and dorsal part of the hippocampus

In order to find out whether the hippocampal subregions were similarly affected by glucocorticoids in their dorsoventral extension, each of the three subregions was divided into a dorsal and ventral part and ChAT activity and the level of 5-HT were measured. The decrease in ChAT activity and in the level of 5-HT induced by AF64A was comparably potentiated by dexamethasone or attenuated after adrenalectomy in both the ventral and dorsal parts of the three subregions. In Figure 3 the results obtained in CA3, the hippocampal area most affected by AF64A, are presented.

Discussion

The present data provide evidence that glucocorticoids play an important role in the susceptibility of the cholinergic septohippocampal pathway to the neurotoxic action of AF64A. Exposure to glucocorticoids in doses that have been used for attaining supraphysiological plasma levels, or concentrations equivalent to those seen after major stressors (according to Sapolsky et al., 1985), exacerbated the AF64A-induced cholinergic deficit, whereas adrenalectomy considerably attenuated this effect of AF64A. The marked effect of adrenalectomy suggests that physiological concentrations of glucocorticoids play a crucial role in, and contribute to, the development of the cholinergic damage caused by AF64A. Short-term exposure to corticosterone was as effective as long-term exposure in increasing the vulnerability of the septohippocampal cholinergic pathway. This early onset of the endangering effect of glucocorticoids is in accordance with previous observations (Sapolsky, 1985b; Johnson et al., 1989; Miller et al., 1989).

Our data show that glucocorticoids exacerbated the AF64A-induced cholinergic lesion in all hippocampal subregions, and adrenalectomy protected all subregions against the action of AF64A. Along the longitudinal axis comparable effects were

Figure 1. Effect of dexamethasone treatment, expressed as percentage change, on the AF64A (1 nmol/ventricle)-induced reduction in ChAT activity in the subregions of the rat hippocampus. The columns (open columns, AF64A/sesame oil, $n = 5$; shaded columns, AF64A/dexamethasone, $n = 7$) represent mean percentage changes $\pm$ SEM (error bars) from the values of the respective vehicle-injected rats ($n = 5$ or 6). The significance of differences between the various groups as calculated from the actual values (two-factor ANOVA with repeated measures and two-tailed Student's t test with α -adjustment according to Bonferroni as a posteriori test) is indicated. *, $p < 0.01$ versus corresponding control group. a, $p < 0.05$; b, $p < 0.01$; versus AF64A/sesame oil.

Table 4. Changes in ChAT activity (nmol/hr/mg tissue) in the rat hippocampus 7 d after intracerebroventricular injection of AF64A (1 nmol/ventricle) in response to short- or long-term treatment with corticosterone

	<i>n</i>	Short-term corticosterone	<i>n</i>	Long-term corticosterone
Sesame oil/vehicle	6	6.33 ± 0.16	5	6.01 ± 0.09
Corticosterone/vehicle	6	6.54 ± 0.09	5	6.08 ± 0.28
Sesame oil/AF64A	5	3.27 ± 0.36**	5	2.55 ± 0.26**
Corticosterone/AF64A	6	2.64 ± 0.12**†	5	1.84 ± 0.15***†

***p* < 0.01 versus corresponding control group.

†*p* < 0.05 versus sesame oil/AF64A treatment.

seen in the dorsal and ventral parts of the hippocampus. Since the CA3 area lacks significant concentrations of type II glucocorticoid receptors or the corresponding mRNA (Van Eekelen et al., 1988; Herman et al., 1989; Stumpf et al., 1989), our observations favor the involvement of the type I mineralocorticoid receptor that is found in all hippocampal subregions. A similar conclusion has been reached by Johnson et al. (1989) concerning the corticosterone-induced sensitization of hippocampal serotonergic neurons to the neurotoxicity of 3,4-methylenedioxymethamphetamine. The occurrence, in primates and rodents, of hippocampal damage associated with prolonged glucocorticoid exposure or aging mainly in the CA3/CA2 region (Landfield et al., 1981; Sapolsky et al., 1985, 1990; Coleman and Flood, 1987) also supports the notion that glucocorticoids act via the type I receptor. Thus, in our experiments, dexamethasone may have either interfered, in the dosage used, at the type I receptor or influenced cholinergic neurons at the level

of the septal area, which contains both types of glucocorticoid receptors.

A variety of factors might be responsible for the endangering effect of glucocorticoids on the septohippocampal cholinergic pathway. (1) Corticosterone, like acute stress or ACTH, induces a rapid activation of the septohippocampal system, including the activity of the HAcHT (Gilad et al., 1985). An increase in

Figure 2. Protection against the AF64A (1 nmol/ventricle)-induced decrease in ChAT activity in subregions of rat hippocampus by bilateral adrenalectomy and reversal of this protective effect by corticosterone replacement. The columns (open columns, sham/AF64A, *n* = 11; shaded columns, adrenalectomy/AF64A, *n* = 10; hatched columns, adrenalectomy/AF64A/corticosterone replacement, *n* = 9) represent mean percentage changes ± SEM (error bars) from the values of the respective control groups (*n* = 7–12). The significance of differences between the various groups as calculated from the actual values (two-factor ANOVA with repeated measures and two-tailed Student's *t* test with α -adjustment according to Bonferroni as a posteriori test) is indicated. *, *p* < 0.01 versus corresponding control group. *a*, *p* < 0.05; *b*, *p* < 0.01; versus adrenalectomy/AF64A.

Figure 3. Influence of bilateral adrenalectomy or dexamethasone treatment on the percentage changes in ChAT activity and 5-HT level in the dorsal and ventral parts of the CA3 subregion induced by the injection of 1 nmol of AF64A/ventricle. Data are mean percentages ± SEM (error bars) of values from the respective vehicle-injected rats. The number of rats per group is given in parentheses. In the control-injected groups four to six rats were used. The open columns represent the corresponding sham-operated/AF64A-treated or sesame oil/AF64A-treated rats, respectively. The significance of differences between the various groups as calculated from the actual values (two-factor ANOVA with repeated measures and Newman-Keuls test with α -adjustment according to Bonferroni as a posteriori test) is indicated. *, *p* < 0.01 versus corresponding control. *a*, *p* < 0.01 versus adrenalectomy/AF64A. *b*, *p* < 0.05; *c*, *p* < 0.01; versus dexamethasone/AF64A.

the velocity of HACHT might therefore allow more AF64A to enter the nerve terminal and thus enhance its toxicity. Although such a mechanism would explain our observation of the exacerbating effect of glucocorticoids, it cannot explain the opposite, that is, protecting, effect of adrenalectomy that also increases the HACHT (Gilad et al., 1985). (2) Recently, Virgin et al. (1991) have pointed out that the glutamate uptake by astrocytes from the synapse is impaired by glucocorticoids, leading to increased synaptic glutamate concentrations; elevated extracellular glutamate concentrations might endanger hippocampal neurons by activating NMDA receptors (Armanini et al., 1990). However, the involvement of glutamate is less likely, since the NMDA antagonist MK 801 did not attenuate the extent of the AF64A-induced cholinergic lesion at physiological glucocorticoid concentrations (Hörtnagl et al., 1991a). (3) Glucocorticoids induce energy deficits (Virgin et al., 1991), which have been claimed to be responsible for the increased neurotoxicity of kainic acid and 3-acetylpyridine (Sapolsky, 1986) and could, in principle, also enhance the toxicity of AF64A. (4) Glucocorticoids have prominent effects on the brain noradrenergic systems (Meyer, 1985). An increase in the firing rate of locus coeruleus neurons and an elevation in NA turnover in brain regions that receive their noradrenergic innervation from the locus coeruleus occur in response to stressful stimuli and to microelectrophoretic application of corticosterone (Thierry et al., 1968; Korset al., 1973; Cassens et al., 1980; Tanaka et al., 1983; Abercrombie and Jacobs, 1987; Avanzino et al., 1987). Stressful stimuli induce a rapid but short-term increase in the activity of tyrosine hydroxylase (Iuvone and Dunn, 1986) and an increase in the extracellular concentration of NA in the hippocampus (Abercrombie et al., 1988; Kalen et al., 1989). Significantly, our results demonstrate that adrenalectomy had the opposite effect, that is, a decrease in NA levels in all subregions of the hippocampus (see Table 2). Since the decrease in NA levels coincided with a 20% reduction in the activity of tyrosine hydroxylase (preliminary results not shown), the activity of the noradrenergic innervation of the hippocampus appears to be depressed. Previous data indicate that the loss of hippocampal cholinergic function induced by AF64A coincides with increased release of NA (Hörtnagl et al., 1989a) and that increased noradrenergic function might in turn enhance the degeneration of cholinergic neurons (Hörtnagl et al., 1989b). Thus, the decreased noradrenergic function resulting from adrenalectomy could at least partly explain the protective effect of adrenalectomy against the neurotoxicity of AF64A. (5) Our finding of possibly increased GABAergic function in response to dexamethasone (as judged from above-normal GAD levels) is unlikely to have been involved in the observed endangering glucocorticoid effect. In contrast to the latter, the enhancement of GAD activity occurred in CA1 and dentate gyrus only and was not observed after corticosterone (indicating a type II glucocorticoid receptor-mediated effect).

On the basis of the above considerations and our present results, we conclude that glucocorticoids may enhance the susceptibility of the septohippocampal cholinergic neurons to AF64A by several mechanisms, including stimulation of HACHT and of noradrenergic activity, and that these effects are mediated by the type I mineralocorticoid receptor. A deficiency in energy supply produced by glucocorticoids might also be involved.

The present findings might have some clinical relevance. There is evidence to show that glucocorticoid secretion increases with age (Landfield et al., 1978; Tang and Phillips, 1978; Sapolsky et al., 1983b; DeKosky et al., 1984) and that the hippocampus

is involved in the feedback control of glucocorticoid secretion in various species, including the primate (Sapolsky et al., 1991). In consequence, glucocorticoids might be involved in the age-dependent decline of the cholinergic function in the basal forebrain cholinergic neurons (Fischer et al., 1987) as well as in the process of cholinergic degeneration occurring in Alzheimer's disease (for review, see Katzman, 1986).

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