EXPERIMENTAL NEUROLOGY 66, 737–744 (1979)

Regional Concentration of Putative Nicotinic–Cholinergic Receptor Sites in Human Brain

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Received June 16, 1979

Various anatomically distinct regions from postmortem human brain were analyzed for α-bungarotoxin (α-BuTX) binding and choline acetyltransferase activity. There was a heterogeneous distribution of both α-BuTX and enzyme activity in the regions studied. Although levels of α-BuTX binding were lower than those reported in other mammalian brain studies, the relative regional concentration was, in general, similar to that of other mammals. High binding activity was noted in the mammillary body, uncus, colliculi, and cortex. Lowest activity was observed in the caudate nucleus and cerebellum. Choline acetyltransferase activities in parallel assays confirmed prior data in human brain.

INTRODUCTION

Alpha-bungarotoxin (α-BuTX), a protein component isolated from the venom of *Bungarus multicinctus*, acts as a high-affinity ligand that has been used to characterize the nicotinic–cholinergic receptor in the peripheral nervous system at the neuromuscular junction (18). A high-affinity α-BuTX-binding component was also demonstrated in the mammalian central nervous system (8, 21). The characteristics of this central α-BuTX-binding site including a high affinity for nicotinic–cholinergic

Abbreviations: α-BuTX—α-bungarotoxin, CAT—choline acetyltransferase.

[1] We thank Dr. Jakob Schmidt for providing native and ^{125}I-α-bungarotoxin, Drs. Carol Petito and Donald Rawlinson for providing assistance in neuropathology, and Isidore Doroski for technical assistance. This research was supported by the Long Island Research Institute and the McKnight Foundation. Drs. Volpe, Francis, and Gazzaniga are at Cornell, and Dr. Schechter is at Stony Brook.

0014-4886/79/120737-08$02.00/0

EXPERIMENTAL NEUROLOGY 66, 745–757 (1979)

Cholinergic Mechanisms and Cataplexy in Dogs

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Received June 20, 1979

Narcolepsy is a disabling neurological disease characterized by excessive daytime somnolence and sudden attacks of partial or complete flaccid paralysis called cataplexy. The disease is known to affect humans as well as dogs. Nineteen dogs diagnosed as narcoleptic were used in this study, which utilized the food-elicited cataplexy test. This test is based on the cataplexy-eliciting effect of food. The results of this study showed that the anticholinesterase physostigmine salicylate (0.05 mg/kg i.v.) and the muscarinic cholinomimetic arecoline hydrochloride (0.15 mg/kg s.c.) significantly increased the amount of cataplexy. Two muscarinic blockers, atropine sulfate (0.1 mg/kg i.v.) and scopolamine hydrobromide (20 μg/kg i.v.), were both effective in significantly reducing the amount of cataplexy. Neostigmine (0.05 mg/kg i.v.), atropine methylnitrate (0.1 mg/kg i.v.), and scopolamine methylnitrate (20 μg/kg i.v.), which do not penetrate the blood–brain barrier, were ineffective. Nicotine (0.03 mg/kg i.v.) and the nicotinic blocker mecamylamine (0.3 and 1 mg/kg i.v.) were also ineffective. The results of this study suggest that central muscarinic cholinergic receptors are critically involved in the mechanism which produces the motor inhibition of cataplexy.

INTRODUCTION

Narcolepsy is an incurable and disabling sleep disorder characterized in its complete form by excessive daytime somnolence, sudden attacks of flaccid paralysis called cataplexy, and the occurrence of sleep paralysis and hypnagogic hallucinations (10).

Abbreviations: FECT—food-elicited cataplexy test, REM—rapid eye movement, EEG—electroencephalogram.

[1] This research was supported by National Institute of Neurological and Communicative Disorders and Stroke grant NS 13211, Research Scientist Development Award MH 05804 to Dr. Dement, and INSERM to Dr. Guilleminault. The authors are indebted to Jon Zirn, Paul Delashaw, and Victoria Neyman, AHT, for their valuable help. Information and reprint requests should be addressed to Dr. Foutz.

0014-4886/79/120745-13$02.00/0

TABLE 1

Stability of Activity in Rat Hippocampus under Postmortem Storage Conditions Described in the Text[a]

Activity	Stored	Fresh	Fresh:stored
Choline acetyltransferase[b]	575 ± 17 (4)	597 ± 18 (4)	0.96
^{125}I-α-bungarotoxin[c]	28.4 ± 1.7 (4)	29.8 ± 1.8 (4)	0.95

[a] Mean ± SE; number of samples in parentheses.
[b] Picomoles per minute per milligram protein.
[c] Femtomoles per milligram protein.

cadavers were kept at 4°C from 90 min after death until autopsy, which was at an average interval after death of 22 h. Tissue samples of 75 to 250 mg were dissected from 15 regions and stored at −80°C until assay for ^{125}I-αBuTX binding or CAT activity 1 to 30 days later.

RESULTS

Stability of Choline Acetyltransferase and ^{125}I-α-Bungarotoxin Activities. The activities obtained from the comparison of freshly obtained rat brain tissue and tissue processed and stored similarly to the human autopsy material are presented in Table 1. The activities of both CAT and ^{125}I-αBuTX were stable under those conditions. The absolute values of both CAT and ^{125}I-αBuTX in the rat hippocampus agreed with previous reports (25, 26).

TABLE 2

Clinical and Pathological Data on Patients with No Neurological Disease

Age, gender	Cause of death	Underlying disease process	Clinical neurologic evaluation	Neuropathologic evaluation
76 M	Sepsis	Perforated colon (diverticulum), membranous nephropathy, renal failure (hemodialysis)	Normal	Unremarkable (1350 g)
55 F	Sepsis	Well-differentiated nodular lymphoma, G.I. hemorrhage	Normal	Unremarkable (1310 g), mild ventricular enlargement
83 F	Pulmonary embolus	Atherosclerotic cardiovascular disease (ASCVD)	Normal	Random anoxic neurons in the cortex, amygdala, and hippocampus (1310 g)
65 F	Heart failure, renal failure	Metastatic adenocarcinoma of the rectum	Normal	Unremarkable (1310 g)

TABLE 3

Clinical and Pathological Data on Patients with Neurological Disease

Age, gender	Cause of death	Underlying disease process	Clinical neurologic function	Neuropathologic evaluation
66 F	Intracerebral hemorrhage	Aplastic anemia, recurrent ovarian cancer	Coma	Massive right frontal lobe hemorrhage (1240 g)
70 M	Pulmonary embolus	Hypertension, multiple cerebral infarcts	Stroke with left hemiparesis	Old right thalamic hemorrhage, était lacunaire (1530 g)
34 M	Increased intracranial pressure, pneumonia	Metastatic malignant melanoma	Focal seizures, right hemisensory defect	Multiple metastatic lesions (1650 g)
72 M	Cardiac arrest	Metastatic prostate cancer, ASCVD, renal failure	Seizures, episodic confusion	Bilateral subdural, widespread anoxic changes (1300 g)
52 F	Respiratory arrest	Hypertension, renal failure (hemodialysis), chronic obstructive pulmonary disease	Seizures, epidosic confusion	Mild ventricular enlargement, widespread anoxic changes (1250 g)
56 M	Cardiac arrest	G.I. perforation, liver failure	Depression, electroconvulsive therapy	Old right caudate infarct, mild anoxic changes (1250 g)

Clinical Pathological Correlations. The clinical history was reviewed in each case: Age, gender, immediate cause of death, underlying medical disorders, antemortem neurological status, and neuropathological evaluation are summarized in Tables 2 and 3.

Of the 10 patients in which postmortem α-BuTX binding was studied, 4 had no neurological disease and no significant neuropathological findings (Table 2). Five patients (Table 3) had significant focal and multifocal structural brain lesions which included an acute massive intracerebral hemorrhage, multiple lacunar infarcts, multiple metastatic lesions, bilateral subdural hematomas, diffuse marked anoxic changes with symmetrically dilated ventricles, old right caudate infarct, and anoxic changes. In all patients with demonstrated focal or multifocal pathology, samples for assay were taken from regions that appeared normal on gross examination; however, there were instances of gross focal damage that prevented a complete sample.

No consistent statistical differences were noted in α-BuTX binding among patients having no neurological abnormalities in contrast to patients with demonstrated focal or multifocal neuropathology (Table 4). There appeared to be no correlation between toxin binding and agonal state.

The regional distributions of α-BuTX binding and CAT activity for different brain regions are presented in Tables 4 and 5. The highest levels of

TABLE 4

Concentration of α-Bungarotoxin Binding Sites from Selected Brain Regions of Normal and Abnormal Cases[a]

Region	Normal cases	Abnormal cases
Mammillary body	17.0 ± 6.4 (3)	14.2 ± 3.2 (5)
Uncus	12.5 ± 6.1 (2)	13.9 ± 5.1 (4)
Inferior colliculus	9.7 ± 2.1 (3)	6.9 ± 0.9 (3)
Superior colliculus	7.4 ± 0.3 (3)	8.3 ± 3.2 (4)
Frontal cortex (olfactory)	7.0 ± 1.6 (3)	9.0 ± 1.7 (5)
Parahippocampal gyrus	6.3 ± 1.1 (3)	7.2 ± 1.0 (4)
Parietal cortex	6.2 ± 2.3 (3)	4.3 ± 1.8 (4)
Amygdala	5.7 ± 0.3 (3)	9.1 (1)
Motor cortex	4.7 ± 2.4 (2)	4.1 (1)
Hippocampus	3.8 ± 1.7 (3)	2.8 (1)
Interpeduncular region	3.6 ± 0.1 (3)	2.5 ± 0.2 (3)
Habenula	3.2 ± 0.3 (2)	2.8 ± 0.5 (2)
Caudate nucleus	1.6 ± 0.5 (3)	
Cerebellar tonsil	0.7 ± 0.2 (3)	0.8 ± 0.4 (4)
Midpons (ventral aspect)	0.3 ± 0.3 (3)	

[a] Number of cases in parentheses; α-bungarotoxin binding in mean ± SE femtomoles per milligram protein.

α-BuTX binding were found in the mammillary nuclei and uncus, with decreasing levels of binding sites in the olfactory cortex, the parahippocampal gyrus, the tectum, and the neocortex. Lowest levels of binding were noted in the caudate nucleus, cerebellar tonsil, and ventral pons. The highest CAT activity was found in the caudate nucleus. High CAT activity was found in the amygdala, the interpeduncular region, and the habenula. The hippocampus, uncus, and olfactory cortex had higher CAT activity than the parietal cortex.

DISCUSSION

The results suggest that in the human brain certain structures within the temporal lobe and diencephalon as well as the tectum contain relatively high concentrations of α-BuTX binding sites, whereas the neostriatum has few nicotinic receptors. These results are largely consistent with findings in the rat (15, 26, 28) and mouse (1). Our findings in human brain differ from those reported in animals chiefly with respect to the low relative concentration of α-BuTX binding sites that we measured in the interpeduncular region and habenula. This difference may have resulted from sampling technique. However, high levels of CAT activity which

TABLE 5

Choline Acetyltransferase Activity from Selected Brain Regions of the Abnormal Cases[a]

Region	Activity
Caudate nucleus	883.0 (1)
Amygdala	225.0 ± 71.4 (3)
Interpeduncular area	132.6 ± 28.1 (3)
Habenula	94.0 ± 11.8 (3)
Uncus	73.2 ± 11.2 (2)
Hippocampus	82.7 ± 16.4 (3)
Frontal cortex (olfactory)	69.3 ± 1.4 (3)
Superior colliculus	69.1 ± 12.1 (3)
Parahippocampal gyrus	64.9 ± 1.7 (3)
Parietal cortex	57.2 ± 6.8 (3)
Motor cortex	49.2 (1)
Mammillary nucleus	42.7 ± 3.1 (3)
Inferior colliculus	36.7 ± 6.4 (3)
Cerebellar tonsil	36.5 ± 5.5 (3)

[a] Number of cases in parentheses. Choline acetyltransferase activity in mean ± SE picomoles per minute per milligram protein.

agree with levels previously found were measured in both those regions (16, 22).

Other reports indicate that the distribution of CAT activity seems to correlate with regional muscarinic receptor activity (31). This correlation may not hold for α-BuTX binding. For example, although certain regions such as the caudate nucleus were reported having high CAT activity and high concentrations of muscarinic receptor (6, 31), the present report indicates a relatively low level of α-BuTX binding. The mammillary body and the inferior colliculus had relatively low CAT activity and high concentrations of α-BuTX binding sites. The present identification of regions with high α-BuTX binding and low CAT activity in contrast to the frequent reports of correlation of regional CAT activity with specific muscarinic receptor suggests that in certain regions CAT localized in synaptic nicotinic structures contributes less to regional CAT levels than CAT localized in synaptic muscarinic cholinergic neurons (5). If the nicotinic–cholinergic receptor were functioning as a postsynaptic receptor on a population consisting largely of "local circuit" neurons (such as the cholinergic neurons in the caudate), one might expect a better correlation between regional CAT and α-BuTX binding.

Recent studies demonstrated reduced CAT activity postmortem in the brains of patients with Alzheimer type dementia and implicated selective

involvement of cholinergic systems in this disorder (6, 24, 30). Muscarinic cholinergic receptor activity was shown to be relatively well preserved (6) despite reduced levels of CAT in Alzheimer-diseased brains. Certain brain regions in which we demonstrated relatively high levels of nicotinic receptor, including the mammillary nuclei, uncus, and parahippocampal gyrus, have been prominently implicated in both the clinical and the experimental literature as essential to cognitive function—particularly memory (13, 24). To date, we have examined two additional patients, one with Parkinson's disease and another with severe senile dementia, and found markedly decreased α-BuTX binding in not only the hippocampus, parahippocampal gyrus, and uncus, but also the superior colliculus. The specificity and etiological significance of these isolated findings remain to be determined in continuing studies.

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