Quebec Cooperative Study
of Friedreich's Ataxia

Brain Neurotransmitter Receptors in Friedreich's Ataxia

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SUMMARY: *The binding of ^{3}H-quinuclidinyl benzilate, a muscarinic cholinergic antagonist, of ^{3}H-dihydroalprenolol, a beta adrenergic antagonist, and of ^{3}H-flunitrazepam, a ligand which labels benzodiazepine receptors, was examined in several regions of control and Friedreich's ataxia (FA) brains. ^{3}H-Quinuclidinyl benzilate binding appeared to increase in the inferior olivory nucleus, anterior and posterior cerebellar vermi but was unaltered in the dentate nucleus and cerebellar hemisphere of FA brain. The binding of ^{3}H-dihydroalprenolol seemed to increase in the inferior olivary nucleus yet was not different from controls in the dentate nucleus, cerebellar hemisphere, anterior and posterior cerebellar vermi of FA brains. ^{3}H-Flunitrazepam binding was slightly lowered in the inferior olivary and dentate nuclei but was unchanged in the other FA brain regions examined. The present study suggests possible trends in neurotransmitter receptor alterations in post-mortem brain tissue of FA patients.*

RÉSUMÉ: *Nous avons examiné dans plusieurs régions de cerveaux contrôles et provenant d'ataxie de Friedreich (AF) la liaison de plusieurs ligands: ^{3}H-quinuclidinyl benzilate (QNB), un antagoniste chlorinergique muscarinique; ^{3}H-dihydroalprenolol (DHA), un antagoniste β-adrénergique; ^{3}H-flunitrazepam (FLU), un ligand qui marque les récepteurs à la benzodiazepine. La liaison de QNB semble augmentée dans le noyau olivaire inférieur, le vermis cérébelleux antérieur et postérieur, mais est inchangée dans le noyau dentelé et les hémisphères cérébelleuses de cerveaux AF. La liaison du DHA semble également augmenté dans le noyau olivaire inférieur, mais ne diffère pas des contrôles dans le noyau dentelé, les hémisphères cérébelleuses et le vermis antérieur et postérieur. Par contre la liaison FLU était légèrement diminuée dans les noyaux olivaires inférieurs et dentelés mais était intacte dans les autres régions examinées de cerveaux AF.*

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INTRODUCTION

Friedreich's ataxia (FA) is an inherited neurological disorder that was first described in 1861 (Friedreich, 1861). Neuropathological studies have revealed that there is severe neuronal degeneration in several regions of the central nervous system. In particular, the sensory fibers of the posterior columns, the spinocerebellar and corticospinal tracts, the brainstem, and cerebellum are most affected in the disease (Wintrobe et al., 1974).

At present, little information is available concerning the possible neurochemical alterations that might be present in the FA central nervous system. Previous studies on such neurological disorders as Parkinson's disease and Huntington's chorea have revealed much information concerning the neurochemical abnormalities of these degenerative diseases (Reisine et al., 1977; Yamamura, 1978). Thus, in a recent study by Huxtable et al., (1978), marked changes in the levels of several amino acids were observed in various FA brain regions which upon pathological examination exhibited extensive neuronal destruction. In the present study, using a limited number of brain samples, we report on the density of muscarinic cholinergic, β-adrenergic, and benzodiazepine receptors in brains obtained post-mortem from two subjects diagnosed as having FA as compared to four patients devoid of any neurological disorders.

SUBJECTS AND METHODS

Post-mortem brain tissue from two patients (average age 19 years) diagnosed as having Friedreich's ataxia were used in this study (Table 1). Four brains were obtained post-mortem from individuals (average

age 40.5 years) devoid of any neurological or psychiatric disorders. The onset (5 years old) of ataxia and death (19 years old) in both FA patients was early in life. Pathological examination of the brain and spinal cord of both FA patients revealed degeneration of the posterior columns, pyramidal and spinocerebellar tracts. Each FA patient had distal peripheral neuropathy with kyphoscoliosis and pes cavus. Both patients had cardiomyopathy.

Attempts were made to obtain age matched controls for this study. Two of the controls patients were between 20-30 years old at the time of death (Table 1). Two older patients were included in the control group since no apparent differences were noted in neurotransmitter receptor binding in the older patient's brain tissue as compared to binding in brain tissue from the younger control patients. Thus, although age matched controls were not used in this study, no significant differences in receptor binding characteristics were observed with age. Therefore, any differences in receptor binding between the FA and control groups is not the result of differences in the ages of the patients at the time of death.

On the average, five hours elapsed between the time of death and freezing (–80° C) of the brain tissue. Before freezing the tissue, the inferior olivary and dentate nuclei, anterior and posterior cerebellar vermi, and cerebellar hemisphere were dissected from each brain. On the day of the experiment, the tissue from each brain region (about 50 mg) was thawed and then homogenized with a Polytron homogenizer (Brinkman, setting 5 for 30 sec.) to make a 5% homogenate in 50 mM sodium-potassium phosphate buffer (pH 7.4). The tissue was washed once by diluting it with 15 ml of buffer and centrifuging it at 48,000 x g for 15 minutes in a Sorvall RC2-B centrifuge. The pellets were resuspended in buffer to make a 5% homogenate. Protein determinations were performed by the method of Lowry et al., (1951).

The ligands employed to measure the various receptor levels were as follows: cholinergic muscarinic receptor, ^{3}H-quinuclidinyl benzilate (^{3}H-QNB); β-adrenergic receptor, ^{3}H-dihydroalprenolol (^{3}H-DHA); and the benzodiazepine receptor, ^{3}H-flunitrazepam (^{3}H-Flu). The interaction of these ligands with their respective receptors has been described elsewhere (Yamamura and Snyder, 1974; Bylund and Snyder; 1976; Speth et al., 1978).

Briefly, the muscarinic cholinergic receptor was assayed in tissue homogenates (50-100 μg protein) which were incubated in 2 ml of 50 mM sodium-potassium phosphate buffer (pH 7.4) for 60 minutes with 100 pM ^{3}H-QNB (29.4 Ci/mmole) in the presence and absence of 1 μM atropine. The reaction was terminated by vacuum filtration through GF/B glass fiber filters, followed by four 5 ml rinses of ice cold buffer. Bound ^{3}H-QNB retained on the filter was extracted in 9 ml of a toluene based scintillation cocktail and radioactivity was monitored in a Searle Mark III liquid scintillation counter. The amount of isotope displaced by atropine is termed specifically bound QNB and is a measure of the number of receptor sites present.

The benzodiazepine receptor was assayed in tissue homogenates (50-100 μg protein) which were incubated for 90 minutes at 0° C in 2 ml of buffer containing 0.5 nM ^{3}H-Flu 87.5 Ci/mmole) in the presence and absence of 1 μM clonazepam. Termination of the reaction was similar to that

TABLE 2

Regional ^{3}H-QNB Binding in FA and Normal Brains

Brain Number	Brain Region				
	Inferior Olivary Nucleus	Dentate Nucleus	Ant. Cere. Vermis	Post. Cere. Vermis	Cere. Hemisphere
FA					
A-77-178	23.8	37.0	48.2	52.2	47.9
A-77-218	40.3	18.9	57.2	59.6	48.3
Average	32.1	28.0	52.7	55.4	48.1
Normal					
A-77-101	—	28.8	31.1	34.2	45.2
A-77-71	15.6	31.8	40.7	33.7	50.1
A-77-51	23.2	—	26.1	31.0	61.1
A-75-144	—	19.2	24.6	38.5	52.3
Average	19.2	26.6	30.6	34.3	52.2

The values are expressed as fmole of ^{3}H-QNB bound per mg protein.
The concentration of ^{3}H-QNB used was 100 pM

TABLE 1

Description of patients from which post-mortem brain tissue was obtained

Brain Number	Interval between Death and freezing (hrs.)	Patient Age (yrs.)	Cause of Death
FA			
A77-178	3.5	19	Heart Failure due to Cardiomyopathy
A77-218	5.5	19	Pneumonia
Average	4.5	19	
Normal			
A77-101	3.0	21	Respiratory Failure
A77-71	9.0	27	Lymphoma
A77-51	2.0	63	Liver cirrhosis
A75-149	5.0	51	Peritonitis
Average	5.0	41	

TABLE 3

Regional ^{3}H-Flunitrazepam Binding in FA and Normal Brains

Brain Number	Brain Region				
	Inferior Olivary Nucleus	Dentate Nucleus	Ant. Cere. Vermis	Post. Cere. Vermis	Cere. Hemisphere
FA					
A-77-178	1.9	1.9	58.8	53.2	55.4
A-77-218	5.4	5.6	42.8	41.1	53.7
Average	3.6	3.7	50.8	47.2	54.6
Normal					
A-77-101	—	4.2	31.4	28.6	28.1
A-77-71	9.0	7.3	56.5	35.2	51.4
A-77-51	5.8	—	52.4	54.2	54.8
A-75-149	—	8.1	60.4	50.6	78.3
Average	7.4	6.5	50.2	42.2	53.2

The values are expressed as fmole of ^{3}H-Flu bound per mg protein.
The concentration of ^{3}H-Flunitrazepam used was 500 pM.

described above. Specific ^{3}H-Flu binding was defined as that binding displaceable by 1 μM clonazepam.

The β-adrenergic receptor was assayed in tissue homogenates (300 μg protein) which were incubated for 30 minutes at 25°C in 2 ml of sodium-potassium phosphate buffer containing 0.25 nM ^{3}H-DHA (58 Ci/mmole) in the presence and absence of 0.1 μM (-)-propranolol. Termination of the reaction was similar to the previously described assays except that the filters were rinsed with buffer maintained at 25°C. Specific ^{3}H-DHA binding was defined as that binding displaceable by 0.1 μM (-)-propranolol.

RESULTS

The results of this study reveal that there may be an increase in ^{3}H-QNB binding in the inferior olivary nucleus and in the anterior and posterior cerebellar vermi of FA brains (Table 2). ^{3}H-QNB binding was unaltered in the FA dentate nucleus and cerebellar hemisphere. ^{3}H-Flu binding appeared to be slightly lowered in the inferior olivary and dentate nuclei yet was unchanged in the anterior and posterior cerebellar vermi and cerebellar hemisphere (Table 3). The binding of ^{3}H-DHA was unaltered in the FA dentate nucleus, anterior and posterior cerebellar vermi, and cerebellar hemisphere while increased in the inferior olivary nucleus (Table 4).

DISCUSSION

The inferior olivary nucleus receives neuronal inputs from the spinal cord, brainstem, and cerebral cortex and is the major source of the climbing fibers which innervate the cerebellum (Noback and Demarest, 1972). Pathological studies revealed that this region is often severely atrophied in FA. In this study, both ^{3}H-QNB and ^{3}H-DHA binding were found to increase while ^{3}H-Flu binding decreased in the FA inferior olive. The increased level of muscarinic cholinergic and β-adrenergic receptors suggests that these receptors are located on nondegenerated cell-types and that decreases in cholinergic and/or noradrenergic neuronal activity might have occurred in the FA inferior olive. The lowered levels of ^{3}H-Flu binding suggests that either benzodiazepine receptors are located on degenerated cells in the inferior olive or that these receptors have become desensitized.

The dentate nucleus is a deep cerebellar nucleus that is severely degenerated in FA. A major inhibitory pathway from the cerebellar cortex (via Purkinje fibers) innervated the dentate nucleus which sends output fibers to the ventral lateral and intralaminar thalamic nuclei as well as the red nucleus (Noback and Demarest, 1972). In the present study, benzodiazepine receptors were slightly depleted in FA dentate nucleus whereas muscarinic cholinergic and β-adrenergic receptors were unaltered. The results suggest that benzodiazepine receptors might be on degenerated cell-types in the FA dentate nucleus, while muscarinic cholinergic and β-adrenergic receptors are on non-degenerated cells.

Interestingly, ^{3}H-QNB binding increased in both the FA anterior and posterior cerebellar vermi. Neither ^{3}H-Flu nor ^{3}H-DHA binding were altered in these same regions. The increased level of muscarinic cholinergic receptors in the FA cerebellar vermi suggests that there may be a loss of

TABLE 4

Regional ^{3}H-DHA Binding in FA and Normal Brains

Brain Number	Brain Region				
	Inferior Olivary Nucleus	Dentate Nucleus	Ant. Cere. Vermis	Post. Cere. Vermis	Cere. Hemisphere
FA					
A-77-178	3.9	10.5	5.8	7.4	6.9
A-77-218	20.2	16.8	11.3	10.0	17.0
Average	12.1	13.6	8.5	8.7	11.9
Normal					
A-77-101	—	9.0	6.8	8.5	5.9
A-77-71	—	14.4	7.5	9.2	9.2
A-77-51	6.6	—	5.5	2.8	4.4
A-75-149	—	10.4	6.7	5.8	14.7
Average	6.6	11.3	6.6	6.6	8.6

The values are expressed as fmole of ^{3}H-DHA bound per mg protein.
The concentration of ^{3}H-DHA used was 250 pM.

cholinergic innervation in the cerebellar vermi of FA brains. Studies measuring the activity of choline acetyltransferase (a marker for cholinergic neurons) are currently in progress in order to determine if such a loss of cholinergic input occurs in the FA cerebellar vermis.

Neuropathological examinations revealed no gross pathological abnormalities in the FA cerebellar hemisphere. In accordance with these findings, no alterations in any of the neurotransmitter receptor levels were detected in this region of FA brains.

In the present study, only a limited number of FA brains were available for analysis. This small sample number prevented statistical comparisons and kinetic analysis of the FA and control groups. Therefore, the results of this study should be viewed with caution. However, this study does reveal trends in neurotransmitter receptor alterations in several FA brain regions. It is hoped that this initial information might promote further examination of the brain neurochemistry of FA.

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