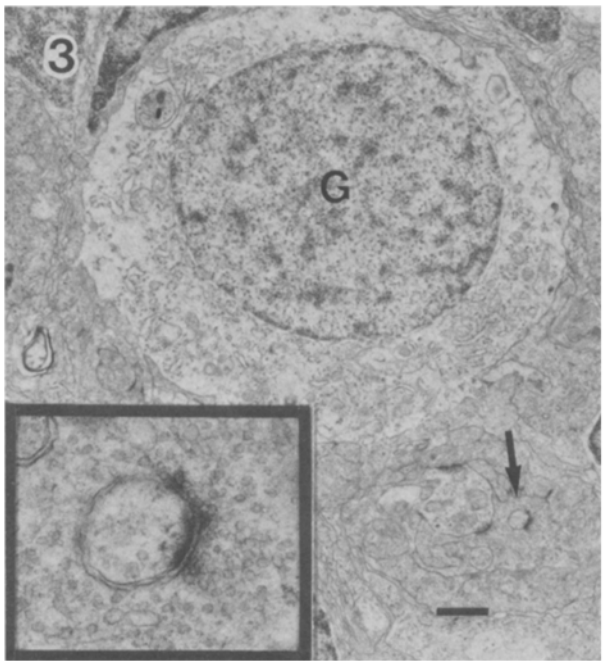

Figure 3. A ganglion cell (G) in the pineal of *S. californiensis* is seen bordering a small neuropil (arrow). Bar indicates 1 μm. Inset: Higher magnification of synapse depicted by arrow.

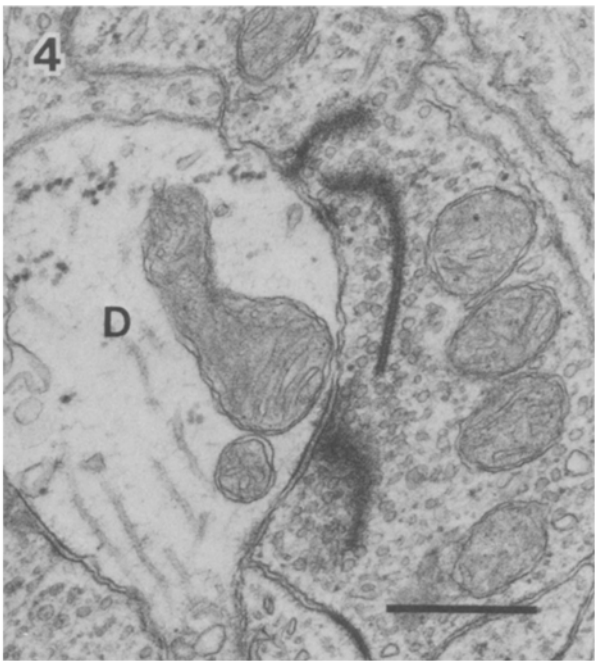

Figure 4. Several goldfish photoreceptor synaptic ribbons contacting a ganglion cell dendrite (D). Bar indicates 0.5 μm.

ship between pineal levels of 5HT and the predominance of synaptic ribbons in the neuropil is unclear with respect to differences in the innervation of the pineal organ in the goldfish and myctophids.

In view of the wide variation in pineal morphology[13], it is noteworthy that the present biochemical findings reflect to some extent the structural diversity reported in the pineal complex of myctophids[5]. Lanternfishes that contained greater amounts of 5HT (*T. mexicanus, S. leucopsaurus, L. ritteri*) have pineal complexes characterized by a prominent central lumen in the end-vesicle and a dorsal sac. By comparison, *T. crenularis* and *S. californiensis*, which tended to have lower levels of 5HT, are species that have compact pineal end-vesicles and lack a dorsal sac.

To summarize, it appears from the present study that structural and biochemical adaptations of the pineal organ to low light levels probably involve both photosensory and neuroendocrine functions in these deep-sea fishes.

1 Falcon, J., Guerlotte, J. F., Voison, P., and Collin, J. P., Neuroendocrinology *45* (1987) 479.
2 McNulty, J. A., Cell Tissue Res. *238* (1984) 565.
3 McNulty, J. A., Gen. comp. Endocr. *61* (1986) 179.
4 McNulty, J. A., and Nafpaktitis, B. G., J. Morph. *150* (1976) 579.
5 McNulty, J. A., and Nafpaktitis, B. G., Am. J. Anat. *150* (1977) 509.
6 McNulty, J. A., Can. J. Zool. *59* (1981) 1312.
7 Omura, Y., and Ali, M. A., Cell Tissue Res. *208* (1980) 111.
8 Korf, H.-W., Cell Tissue Res. *174* (1976) 475.
9 McNulty, J. A., Cell Tissue Res. *172* (1976) 205.
10 van Veen, Th., Laxmyr, L., and Borg, B., Gen. comp. Endocr. *46* (1982) 322.
11 Meissl, H., and George, R., Vision Res. *24* (1984) 1727.
12 Falcon, J., Geffard, M., Juillard, M. T., Steinbusch, H. W. M., Sequela, P., and Collin, J. P., J. Histochem. Cytochem. *32* (1984) 486.
13 Vollrath, L., The Pineal Organ, Handbuch der Mikroskopischen Anatomie des Menschen, VI/7. Springer-Verlag, Berlin 1981.

0014-4754/88/090740-03$1.50 + 0.20/0

Effect of diabetes on the enzymes of the cholinergic system of the rat brain[1]

Z. Z. Wahba and K. F. A. Soliman[2]

College of Pharmacy and Pharmaceutical Sciences, Florida A & M University, Tallahassee (Florida 32307, USA)
Received 3 March 1988; accepted 14 June 1988

Summary. Choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities were determined in several brain regions of normal and streptozotocin-induced diabetic rats. The diabetic rats exhibited significant increase in ChAT activity ($p < 0.05$) in all brain regions studied except for the cortex and the midbrain. Meanwhile, the diabetes condition was associated with significant increase ($p < 0.05$) in AChE activity of the bulbus olfactorius, medulla oblongata and cerebellum. These data suggest that uncontrolled diabetes is associated with significant alterations in the brain cholinergic systems.
Key words. Choline acetyltransferase; acetylcholinesterase; brain; diabetes.

Involvement of the nervous system in persons with diabetes has long been recognized. Until the middle of the 19th century, diabetes itself was ascribed to a disorder of the central nervous system[3]. Diabetes mellitus has been reported to be accompanied by a number of behavioral and hormonal abnormalities, including reduced locomotor activity[4] and impaired hypothalamic function[5]. The peripheral nerve, brain and spinal cord may all be damaged in long-standing diabetes mellitus[6-8]. The morphologic basis of diabetic autonomic neuropathy appears to be both axonal damage and segmented demyelination[9]. Some evidence suggests that the disordered glucose metabolism may play a role in diabetic neuropathy[10]. Impaired axonal transport produces numerous biochemical abnormalities in the axon[9]. Neuronal degeneration in the brain and spinal cord may also appear[6,11]. The observations that in patients with newly diagnosed diabetes and in laboratory animals with experimental diabetes neurological deficits were found to be either preventable in laboratory animals[12] or partially correctable in humans through insulin treatment, support the concept that an early, reversible impairment in neuronal function in diabetes is due to biochemical changes that precede structural abnormalities of the neurons[3]. Diabetes was found to be associated with changes in somatic sensations which involve the thalamus, cerebral cortex and cerebellum. Pain loss, impaired touch perception and decreased position sense all have been documented in the diabetic patient[14]. Diabetes is also associated with autonomic neuropathy. A common autonomic neuropathy of a cholinergic component includes diabetic diarrhea, impotence, sweating and bladder dysfunction[15,16].

An equally important aspect of the neuropathological effect of diabetes is, undoubtedly, the neurochemical changes that may accompany morphologic alteration in the nervous system. Several recent studies have addressed the issue of whether or not diabetes affects neurotransmitter substances and associated enzymes in the nervous system[17-19]. Such studies obviously could contribute to our understanding of the neuropathological effects of diabetes. The enzymes of the cholinergic system, choline acetyltransferase (ChAT, E.C. 2.3.1.6) and acetylcholinesterase (AChE, E.C. 3.1.1.7) have been used as markers of cholinergic activity. Therefore, the present investigation was designed to study the effects of diabetes on the cholinergic enzyme activity in the rat brain.

Materials and methods. Male Sprague-Dawley rats obtained from Southern Animal Farms (Prattville, AL) and weighing 150–180 g were used in this study. To induce diabetes, the aqueous streptozotocin solution in citric acid buffer (pH 4.4) was injected i.v. in a dose of 40 mg/kg, 14 weeks prior to sacrificing the animals by decapitation. Trunk blood was collected in heparinized tubes, centrifuged, and plasma was separated and assayed for glucose using Beckman's glucose analyzer (Fullerton, California). The brain from each animal was quickly removed and dissected on ice into cerebral cortex, bulbus olfactorius, midbrain, medulla oblongata, hippocampus, hypothalamus, cerebellum, pons and thalamus. Each brain region was homogenized (1% w/v) in ice-cold 0.5 M phosphate buffer pH 7.0, and ChAT activity was expressed as micromoles of COASH formed per minute per milligram of tissue[20]. For the determination of AChE activity, each brain region was homogenized (1% w/v) in ice-cold

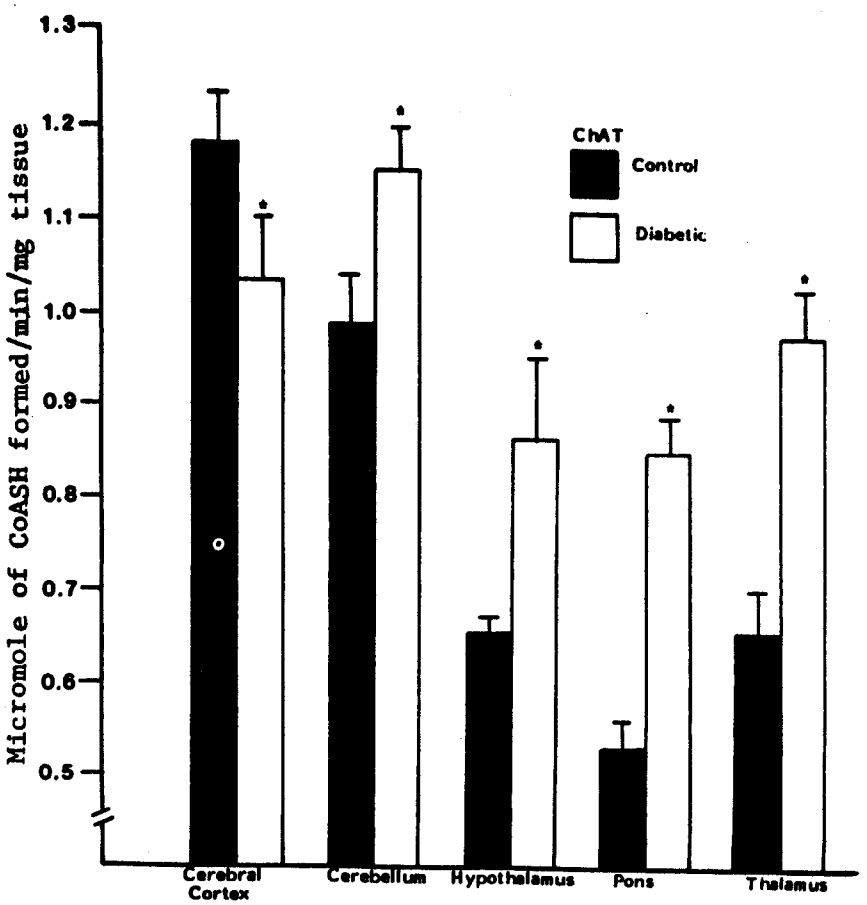

Figure 1. The effect of 14 weeks streptozotocin-induced diabetes (40 mg/kg i.v.) on the activity of ChAT in different regions of rat brain. Each bar represents the mean value ± SEM for 8 rats.

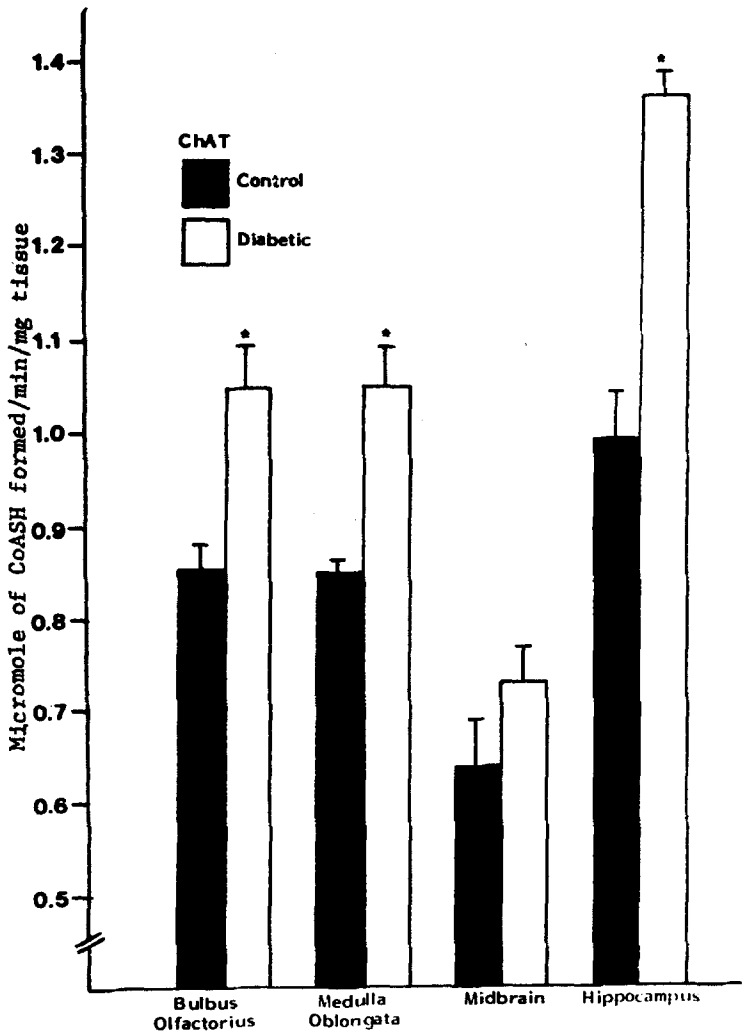

Figure 2. The effect of 14 weeks streptozotocin-induced diabetes (40 mg/kg i.v.) on the activity of ChAT in different regions of rat brain. Each bar represents the mean value ± SEM for 8 rats.

0.1 M phosphate buffer pH 7.4 containing 0.1% triton X-100 (Sigma). AChE activity in the homogenate was then determined by a spectrophotometric method[21] and expressed as micromoles of substrate acetylthiocholine iodide (Sigma) hydrolyzed per minute per gram of tissue. Data were statistically analyzed using a Student's t-test.

Results. One day following the injection of streptozotocin, experimental animals showed intense glucosuria. All control animals showed no glucose in urine. Glucose concentration of the diabetic and control rats measured at the time of sacrificing averaged 535.9 mg% with SEM of 43.2 and 123.1 mg% with SEM of 35.8, respectively. The difference between the diabetic group and control group in plasma glucose was highly significant ($p < 0.01$).

During the course of the experiment, the diabetic animals presented a retarded weight gain. At the time of sacrificing, the body weights of diabetic rats averaged 251.8 g with SEM of 42.8 g while the control group body weight averaged 394.5 g with SEM of 51.3 g. The difference in body weight between the diabetic and control group was highly significant ($p < 0.01$).

Figures 1 and 2 show the effects of diabetes on ChAT activity of the various rat brain regions studied, compared to the control animals. Significantly higher values of ChAT activity in the cerebellum ($p < 0.05$), hypothalamus ($p < 0.05$), pons ($p < 0.01$), thalamus ($p < 0.01$), bulbus olfactorious ($p < 0.05$), medulla oblongata ($p < 0.05$), and hippocampus ($p < 0.01$) were observed in diabetic rats, 14 weeks after the injection of streptozotocin. This was accompanied by a significant decline in ChAT activity of the cerebral cortex ($p < 0.05$), and no significant change in midbrain ChAT activity.

Figures 3 and 4 depict AChE activity in various brain regions of streptozotocin induced-diabetes and control rats. Induction of diabetes resulted in a significant increase of AChE activity in the cerebellum ($p < 0.05$), bulbus olfactorius ($p < 0.05$), and medulla oblongata ($p < 0.05$). A significant decline of AChE activity in the hypothalamus ($p < 0.01$), pons ($p < 0.01$) and thalamus ($p < 0.05$) and no significant changes in AChE activity in the cerebral cortex, midbrain and hippocampus were observed in diabetic rats as compared to controls.

Discussion. The results of the present study indicate that diabetes affected the level of the cholinergic enzymes in most of the brain regions studied. The significant increase in both ChAT and AChE activity in the bulbus olfactorius, medulla

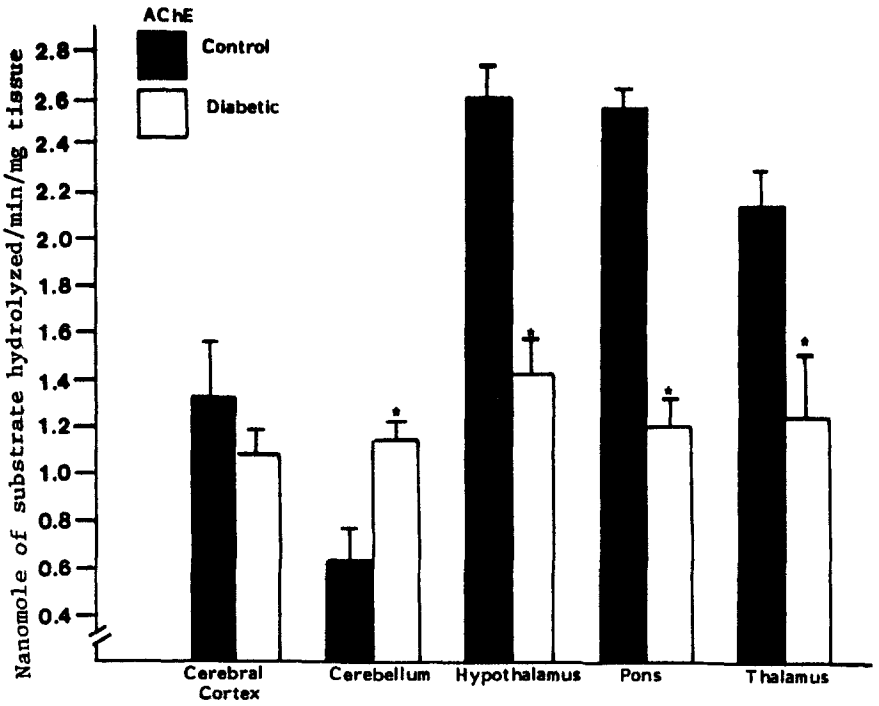

Figure 3. The effect of 14 weeks streptozotocin-induced diabetes (40 mg/kg i.v.) on the activity of AChE in different regions of rat brain. Each bar represents the mean value ± SEM for 8 rats.

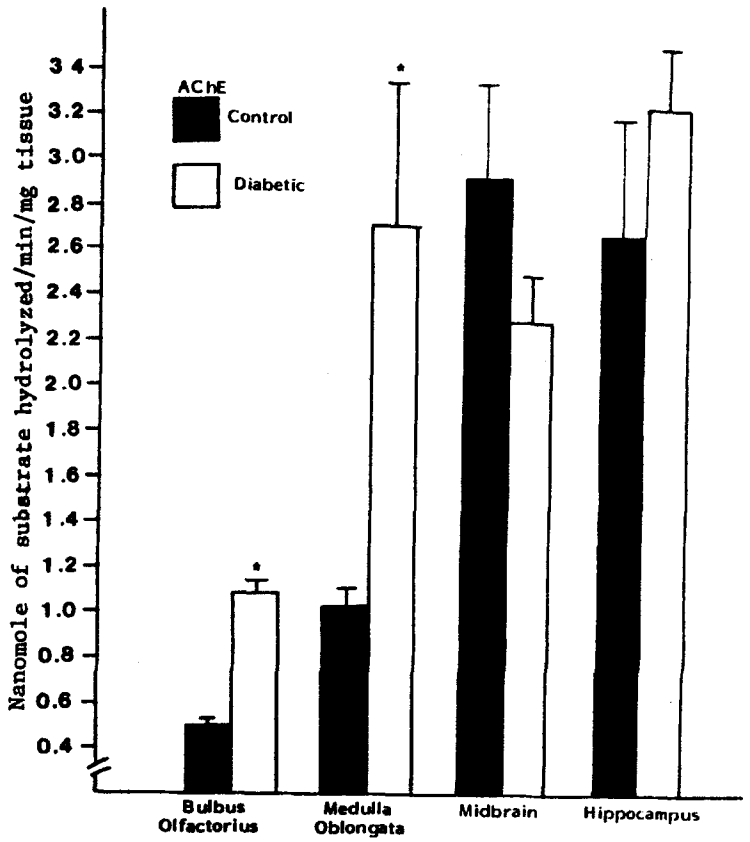

Figure 4. The effect of 14 weeks streptozotocin-induced diabetes (40 mg/kg i.v.) on the activity of AChE in different regions of rat brain. Each bar represents the mean value ± SEM for 8 rats.

oblongata and cerebellum may reflect an increased synthesis of these enzymes, possibly due to changes in retrograde axonal transport, which normally delivers information to the nerve body concerning the state of the axon and its terminals under diabetic conditions[9]. A close correlation between these two enzymes within subsectors of the hippocampal formation with certain pathological implications has been reported[22]. However, the close correspondence between these two enzymes does not hold everywhere in the brain. For example, our data shows a significant elevation of ChAT activity in the hypothalamus, thalamus and pons, while AChE activity was decreased in these brain regions.

The alterations in the cholinergic enzymes associated with diabetes in the brain may, in part, explain the abnormality in hypothalamic function[5], locomotor activity[4], intestinal motility[15], male penile erection, sweating and bladder function[16].

It has been known that diabetes has a profound effect on the brain chemistry[23, 24], particularly its effects on brain neurotransmitters and associated enzymes. For example, a reduction in brain serotonin synthesis rate in diabetic rats has been reported[24], and diabetes resulted in changes in the monoamine oxidase activity of the rat brain[18]. Recently, we have shown that diabetes was associated with concomitant changes in brain beta endorphin and brain insulin[17]. In another study, a decreased rate of dopamine synthesis in the brain of streptozotocin-diabetic rats was reported[19]. More recently, it was found that streptozotocin-induced diabetes was associated with an alteration in the metabolism of brain monoamines[25]. The above studies provide evidence that experimentally induced diabetes may affect the synthesis and/or metabolism of brain neurotransmitter substances. Of particular interest in the present findings is the ChAT activity. ChAT is generally accepted as a specific marker for cholinergic structure in the brain[26]. Thus valuable information can be obtained in studying the activity of this enzyme. In the present investigation ChAT activity is enhanced in most brain regions in diabetic animals. This effect may represent a compensatory mechanism in cholinergic transmission in the brain of diabetic rats.

1 This work was supported by grants from the National Aeronautics and Space Administration (NSG 2183 and NAG-2-411), a grant from the National Institutes of Health (NIH Grant RR0811) and a grant from the Division of Research Resources, National Institutes of Health (NIH Grant RR03020).

2 To whom requests for reprints should be addressed.
3 Locke, S., and Tarsy, D., in: Joslin's Diabetes mellitus, 12th edn. pp. 665–685. Eds A. Marble, L. P. Krall, R. A. Christlieb and J. S. Soeldner. Lea & Febiger, Philadelphia 1985.
4 Marshall, J. F., Friedman, M. I., and Haffner, T. G., Brain Res. *111* (1976) 428.
5 Tannenbaum, G. S., Endocrinology *108* (1981) 76.
6 Colby, A. O., Diabetes *14* (1965) 424.
7 Dahlin, L. B., Meiri, K. F., McLean, W. C., Rydevik, B., and Sjostrand, J., Diabetologia *29* (1986) 181.
8 Powell, H., Knox, D., Lee, S., Sharters, A. C., Orloff, M., Garrett, R., and Lampert, P., Neurology (Minneap.) *27* (1977) 60.
9 Niakan, E., Harati, Y., and Comstock, J. P., Metabolism *35* (1983) 224.
10 Brown, M. J., Dyck, P. J., McClean, G. E., Sima, A. A. F., Powell, H. C., and Porte, D. Jr, Diabetes *31* (1982) 65.
11 Carson, K. A., Bassen, E. H., and Hanker, J. S., Neuropath. appl. Neurobiol. *6* (1980) 361.
12 Winegrad, A. I., and Green, D. A., N. Engl. J. Med. *295* (1976) 1416.
13 Ward, J. D., Barnes, C. G., and Fisher, D. J., Lancet *1* (1971) 428.
14 Waxman, S. G., and Sabin, T. D., Arch. Neurol. *38* (1981) 46.
15 McNally, E. F., Reinhard, A. E., and Schwartz, P. E., Am. J. Dig. Dis. *14* (1969) 163.
16 Katz, L. A., and Spiro, H. M., N. Engl. J. Med. *275* (1966) 1350.
17 Kolta, M. G., Soliman, K. F. A., and Williams, B. B., Hormone Res. *23* (1985) 112.
18 Mayanil, C. S., Kazmi, S. M., and Baquer, N. Z., J. Neurochem. *38* (1982) 179.
19 Trulson, M. E., and Himmel, C. D., J. Neurochem. *40* (1983) 1456.
20 Chao, L. P., and Wolfgram, F., Analyt. Biochem. *46* (1972) 114.
21 Ellman, G. L., Courtney, K. D., Andres, V. F., and Featherstone, R. M., Biochem. Pharmac. *7* (1961) 88.
22 Fonnum, F., J. Neurochem. *17* (1970) 1037.
23 Reske-Nielsen, E., and Lundbaek, K., Acta neurol. scand. *39* (Suppl. 14) (1963) 273.
24 Reske-Nielsen, E., Lundbeak, K., and Rafaelsen, O. J., Diabetologia *1* (1965) 233.
25 Bitar, M., Kolu, M., Rapoport, S. I., and Linnoila, M., J. Pharmac. exp. Ther. *236* (1986) 432.
26 Nieoullon, A., and Dusticier, N., Neuroscience *6* (1981) 1633.

0014-4754/88/090742-05$1.50 + 0.20/0

Molecular forms of dopamine beta-hydroxylase in rat superior cervical ganglion and adrenal gland

N. H. Fraeyman, E. J. Van de Velde and F. H. De Smet

Heymans Institute of Pharmacology, University of Ghent, Medical School, De Pintelaan 185, B-9000 Ghent (Belgium)
Received 7 January 1988; accepted 10 June 1988

Summary. Dopamine beta-hydroxylase (DBH) enzyme activity was associated in rat superior cervical ganglion with tetrameric DBH-A (294,000 D) and dimeric DBH-B (147,000 D) and in rat adrenal gland with DBH-A and a novel molecular form of DBH, defined as DBH-C, with a molecular weight of 125,000 D. Pretreatment of the rats with cycloheximide markedly reduced DBH activity without altering the molecular heterogeneity.
Key words. Dopamine beta-hydroxylase; molecular forms; enzyme activity; gradient centrifugation; rat.

It has been reported that the enzymatic activity of DBH, determined in non-denaturating conditions, is attributable to multiple molecular forms with different molecular weights. Thus, in sera of different species, tetrameric and dimeric form of the same subunit of DBH, have been described[1–3]. Studies in tissues, however, are complicated by