Neuroscience Letters, 14 (1979) 101—104

LOCUS CERULEUS SOMATA CONTAIN BOTH ACETYLCHOLIN ESTERASE AND NOREPINEPHRINE: DIRECT HISTOCHEMICAL DEMONSTRATION ON THE SAME TISSUE SECTION

ALBERTO ALBANESE and LARRY L. BUTCHER

Department of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024 (U.S.A.)

(Received June 12th, 1979)
(Accepted June 15th, 1979)

SUMMARY

In the same brain section stained first for catecholamines and then for acetylcholinesterase (EC 3.1.1.7), it was found that neuronal somata in locus ceruleus containing norepinephrine also contained the cholinergic degradative enzyme. Cell body shapes were fusiform, pyramidal, round, or oval. Maximum soma extent ranged from 10—40 μm, with the proportion of large diameter neurons increasing, and the smaller diameter cells decreasing, from rostral to caudal cerulear levels. Medium-sized neurons were roughly constant throughout the nucleus. Acetylcholinesterase may be associated with cerulear somata and proximal processes containing norepinephrine to inactivate a cholinergic input to that structure.

The localization of acetylcholinesterase (AChE, EC 3.1.1.7) within and/or on norepinephrine-containing neurons in locus ceruleus (group A6 of Dahlström and Fuxe [6]) has been deduced from several experimental observations [1,8]. First, the morphologies and cytoarchitectural organization of norepinephrine and AChE somata in locus ceruleus are similar, if not identical, to one another [1,8]. Second, retrograde degeneration of AChE-containing cell bodies in locus ceruleus is produced in rats having unilateral radio-frequency lesions in both the cerebellum and dorsal tegmental bundle [1], two areas possessing norepinephrine-containing afferents from locus ceruleus. And third, injection of colchicine into the dorsal tegmental bundle and cerebellum produces accumulation of AChE within processes, probably axons, deriving from locus ceruleus neuronal somata [1].

Address correspondence to: Dr. Larry L. Butcher, Department of Psychology, University of California, 405 Hilgard Avenue, Los Angeles, CA 90024, U.S.A.

The preceding evidence is not direct, however, and, in this report, we present histochemical data that demonstrate on the same brain section the association of AChE with norepinephrine somata in locus ceruleus.

Female Sprague—Dawley rats weighing 200—300 g were used. They were injected first with Nialamide (100 mg/kg, i.p.) and then with bis(1-methylethyl)phosphorofluoridate (DFP, 0.8 mg/kg, i.m.) six and four hours, respectively, prior to being sacrificed. The brain was rapidly removed from the cranial cavity, blocked, mounted on a brass specimen holder, and frozen in a cryostat at −25°C. Transverse sections, 8 μm thick, were processed for monoamines according to the glyoxylic acid procedure of de la Torre and Surgeon [7].

After examination and photography of monoamine histofluorescence, the same brain sections were stained for AChE according to the regimen described in Butcher and Bilezikjian [2], Butcher and Hodge [3], and Butcher and Marchand [4]. As reported previously [4], the DFP used in the pharmacohistochemical regimen for AChE [2] did not influence catecholamine fluorescence, at least as assessed histochemically. Similarly, the use of Nialamide, which enhances catecholamine fluorescence in neuronal somata, did not influence subsequent staining for AChE.

Comparison of fluorescence patterns and AChE staining on the same brain sections revealed that norepinephrine somata in locus ceruleus contained AChE (compare Fig. 1A with 1B). Such neuronal somata were fusiform, pyramidal, oval, or round. The fusiform cell bodies possessed two processes staining for both AChE and norepinephrine, one of which was more prominent than the other; the pyramidal, oval, and round somata were multipolar. The deposits of russet-colored reaction product representing AChE loci were organized in a peri-nuclear array; norepinephrine staining was similarly organized and followed patterns of AChE staining (compare Fig. 1A with 1B). All norepinephrine somata in locus ceruleus appeared to contain AChE.

Three categories of cerulear neurons were found on the basis of cell size. Medium-sized neurons had some dimensions within the range 20—30 μm. The small neurons (soma dimensions: 10—20 μm) were found primarily in the dorsal locus ceruleus, and the large neurons (soma dimensions: 30—40 μm) were observed mostly in ventral portions. The proportion of large AChE neurons decreased from 32% of the total at caudal levels to 6.5% at rostral regions of locus ceruleus; at the same caudo-rostral loci the proportion of small cells increased from 23% of the total caudally to 48% rostrally. The proportion of medium-sized neurons was essentially constant throughout the caudo-rostral extent of the nuclear mass. Some neurons, approximately 33% of the total regardless of soma shape or size, stained lightly for AChE and norepinephrine, whereas the remaining cells stained heavily.

Although Sladek and Walker [11] report the presence of a few 5-hydroxytryptamine-containing cerulear somata in neonatal and juvenile macaque brain, they did not observe serotonin somata in the locus ceruleus of the

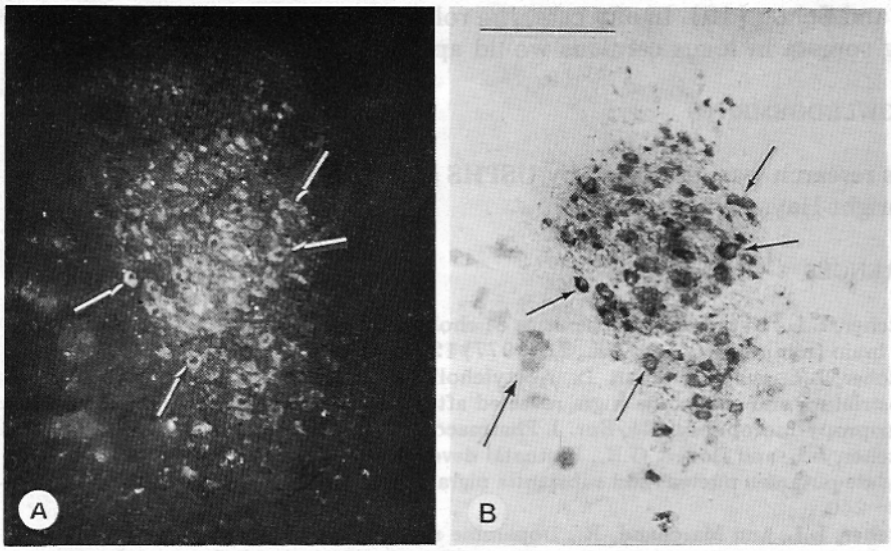

Fig. 1. Demonstration in rostral locus ceruleus of norepinephrine (A) and acetylcholinesterase (B) on the same brain section. Small arrows in A and B point to the same neuronal somata. Large arrow in B points to cell body in the mesencephalic nucleus of cranial nerve V. Scale = 200 μm.

adult monkey, and we did not find evidence for such cells in adult rat brain treated with hot formaldehyde gas to demonstrate monoamines [6,11]. Furthermore, no somata were observed that stained only for AChE and not simultaneously for norepinephrine. Putative 5-hydroxytryptamine-containing cell bodies in locus ceruleus may not contain AChE, therefore.

It is open to speculation why norepinephrine-containing neurons in locus ceruleus should contain AChE, but one hypothesis is that neurons containing acetylcholine as a neurotransmitter project to locus ceruleus, and AChE is postsynaptically localized in and/or on norepinephrine-containing cerulear somata to inactivate released acetylcholine. This proposal derives support from the observations of Kuhar et al. [9] that iontophoretic application of acetylcholine increases spontaneous firing rates of locus ceruleus cells. When physostigmine is coadministered with acetylcholine, there is a potentiation of the effects of the cholinester [9]. Furthermore, Cheney et al. [5] have shown that locus ceruleus contains both acetylcholine and choline acetyltransferase. Lewis and Schon [10], however, on the basis of electron microscopic data, concluded that there was no evidence for cholinergic mechanisms operating in the locus ceruleus because, although locus ceruleus contained larged amounts of AChE, the enzyme could not be detected in the synaptic cleft or associated with presynaptic membranes. As pointed out by Kuhar et al. [9], however, such negative data should be interpreted with caution owing to possible methodologic difficulties with the procedure of

Lewis and Schon [10]. In any case, the role of AChE in norepinephrine-containing somata in locus ceruleus would appear worthy of further study.

ACKNOWLEDGEMENTS

This research was supported by USPHS grant NS 10928 to L.L.B. A.A. is a Fulbright-Hays Scholar.

REFERENCES

1 Butcher, L.L., Nature and mechanisms of cholinergic-monoaminergic interactions in the brain (minireview), Life Sci., 21 (1977) 1207—1226.
2 Butcher, L.L. and Bilezikjian, L., Acetylcholinesterase-containing neurons in the neostriatum and substantia nigra revealed after punctate intracerebral injection of di-isopropyfluorophosphate, Eur. J. Pharmacol., 34 (1975) 115—125.
3 Butcher, L.L. and Hodge, G.K., Postnatal development of acetylcholinesterase in the caudate-putamen nucleus and substantia nigra of rats, Brain Res., 106 (1976) 223—240.
4 Butcher, L.L. and Marchand, R., Dopamine neurons in pars compacta of the substantia nigra contain acetylcholinesterase: Histochemical correlations on the same brain section, Eur. J. Pharmacol., 52 (1978) 415—417.
5 Cheney, D.L., LeFevre, H.F. and Racagni, G., Choline acetyltransferase activity and mass fragmentographic measurement of acetylcholine in specific nuclei and tracts of rat brain, Neuropharmacology, 14 (1975) 801—809.
6 Dahlström, A. and Fuxe, K, Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons, Acta physiol. scand., 62, Suppl. 232 (1965) 1—55.
7 de la Torre, J.C. and Surgeon, J.W., A methodological approach to rapid and sensitive monoamine histofluorescence using a modified glyoxylic acid technique: The SPG method, Histochemistry, 49 (1976) 81—93.
8 Knight, D.P., Histochemical demonstration of catecholamines and acetylcholine esterase in the same cell bodies in the locus coeruleus (rat hind brain), Proc. Royal Microscop. Soc., 6 (1970) 26—27.
9 Kuhar, M.J., Atweh, S.F. and Bird, S.J., Studies of cholinergic-monoaminergic interactions in rat brain. In L.L. Butcher (Ed.), Cholinergic-Monoaminergic Interactions in the Brain, Academic Press, New York, 1978, pp. 211—227.
10 Lewis, P.R. and Schon, F.E.G., The localization of acetylcholinesterase in the locus coeruleus of the normal rat after 6-hydroxydopamine treatment, J. Anat. (Lond.), 120 (1975) 373—385.
11 Sladek, J.R., Jr. and Walker, P., Serotonin-containing neuronal perikarya in the primate locus coeruleus and subcoeruleus, Brain Res., 134 (1977) 359—366.