An Autoradiographic Study of the Mouse Carotid Body Using Tritiated Leucine, Dopa, Dopamine and ATP with Special Reference to the Chief Cell as a Paraneuron*

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Received August 5, 1976

Summary. A comparative autoradiographic study on the uptake and intracellular localization of $^{3}$H-leucine-, $^{3}$H-dopa-, $^{3}$H-dopamine- and $^{3}$H-ATP-derived radioactivity was performed in the mouse carotid body to investigate the metabolic features of the chief cell as a paraneuron.

$^{3}$H-leucine-derived radioactivity representing recently synthesized peptides was demonstrated in all kinds of cells in the carotid body and surrounding area. The chief cell was less radioactive than the nerve cell in the superior cervical ganglion. In the electron microscope autoradiography, no accumulation of radioactivity could be demonstrated either in the Golgi area of the chief cell, where the membrane-bound particles were probably formed, nor in the periphery of the cell, where they were stored before their release.

Incorporation of $^{3}$H-dopa-derived radioactivity representing recently synthesized catecholamines was specific to the chief cell, mast cell, and nerve cell in the superior cervical ganglion. In the chief cell the distribution of radioactivity was roughly identical with that of the large dense-cored vesicles.

Striking accumulations of $^{3}$H-dopamine-derived radioactivity were demonstrated in the adrenergic nerve terminals in the perivascular space and the glomus complexes of the carotid body. Not all of the chief cells incorporated the $^{3}$H-dopamine-derived radioactivity.

$^{3}$H-ATP-derived radioactivity was demonstrated in all kinds of cells in the carotid body and surrounding tissues. In the chief cell, as in other kinds of cells, the highest radioactivity was seen in the nucleus.

The present results suggest that, if the large dense-cored vesicles and/or small synaptic vesicles in the chief cell, like those membrane-bound particles in other paraneurons, contain peptides, monoamines and ATP, the turnover of these products as secretory materials is much slower in this cell than in such endocrine paraneurons as adrenal chromaffin cells and gut endocrine cells.

The chief cell of the mouse carotid body contains two types of membrane-bound particles in the cytoplasm (KOBAYASHI and UEHARA, 1970). The first ones called large dense-cored vesicles or dense-cored vesicles are 80–90 nm (KOBAYASHI and UEHARA, 1970) or 80–120 nm (Böck, 1973) in diameter. These vesicles are dispersed throughout the cytoplasm, but frequently tend to accumulate and appose the cell membrane (Böck, 1973; Böck and GORGAS, 1976). The second ones, called small synaptic vesicles, are 30–40 nm in diameter aggregating particularly in the region where the nerve terminals are in apposition. Considering the size, uniformity and peculiar localization of the small synaptic vesicles, KOBAYASHI and UEHARA (1970) interpreted the assemblages of these vesicles and desmosome-like membrane thickenings between

* This work was supported by a grant from the Ministry of Education of Japan
the chief cell and the nerve terminal as the site of chemosensory transmission. On
the other hand, the large dense-cored vesicles are supposed to be of endocrine nature,
since there are substantial ultrastructural data suggesting the occurrence of their
exocytotic release almost anywhere on the surface of the chief cell (Biscoe and
Steibbens, 1966; Blümcke, Rode and Niedorf, 1967; Kobayashi, 1968; Böck, Stockinger
The presence of the two types of subcellular particles was reported first in the
mouse, then in the guinea pig, Uroloncha domestica, Hynobius nigrescens by Kobayashi
(1971b), in the rabbit by Verna (1971, 1973), in the rat by McDonald and Mitchel
(1975a, b) and in domestic fowl by King and his co-workers (Hodges et al., 1975; King
et al., 1975).
It seems essential for the elucidation of the functional significance of the chief
cell in the carotid body chemoreceptor, to know the chemical composition of the two
populations of cytoplasmic particles and the mechanism of their formation, matura-
tion and release.
There is a good deal of evidence that the chief cell of the carotid body shares a
number of cytological, cytochemical, ultrastructural and embryological features with
a wide spread system of peptide-secreting endocrine cells (Capella and Solcia, 1971;
the cells of this system are not typical neurons but rather endocrine or sensory cells;
they all contain neurotransmitters or suspected transmitters; they all have mem-
brane-bound particles in the cytoplasm; and they are either proved or strongly
suspected to be neuroectodermal in origin. Fujita (1976) proposed the concept of
paraneurons for the members of this whole family of modified nerve cells.
Biochemical analyses in some of the paraneurons like adrenal chromaffin cells
and pancreatic islet cells, have revealed that the membrane-bound particles contain
more or less peptides, monoamines and ATP (Hillarp, 1958; Schumann, 1966; Leitner
et al., 1975). These substances probably form a storage complex or supermolecule
under the presence of divalent cations (Winkler and Hörtnagl, 1973).
It has been proposed that not only the chemical composition but also the origin
and fate of the membrane-bound particles is essentially shared by all the varieties
of paraneurons (Fujita, 1976; Kobayashi, 1976). They are formed in the Golgi area
where they contain mainly peptides, and later, during the maturation of the particles,
icorporate ATP and monoamines from the cytosol to become ready at the cell peri-
iphery to be released by the process of exocytosis in response to the nervous or humoral
stimuli to the cell (Kobayashi, 1976).
Thus, it is speculated that the carotid body chief cell, like other paraneurons,
might contain in its dense-cored vesicles and small synaptic vesicles, peptides, mono-
amines and ATP. Worthy of note is that, a combined occurrence of catecholamines
and ATP in the carotid body was suggested by Torrance about a decade ago (see
discussion by Torrance in the referred book, p. 113). However, this is obviously a
working hypothesis, and needs to be confirmed by a considerable amount of further
research.
The present autoradiographic study in the mouse carotid body was designed in
the hope that autoradiography might provide some clue with regard to the subcellular
localization and dynamics of newly synthesized peptides, catecholamines and ATP.
in the chief cell. The results will be compared especially with those in the adrenal chromaffin cells and gut endocrine cells on which similar, but more extensive autoradiographic studies have recently been performed by us using tritiated amino acids and amine precursors (KOBAYASHI, 1975, 1976; KOBAYASHI and SASAGAWA, 1976; COUPLAND and KOBAYASHI, 1976; COUPLAND, KOBAYASHI and CROWE, 1976; COUPLAND, KOBAYASHI and KENT, 1976).

**Paraneuron**

The term and concept of paraneuron was proposed by Prof. T. FUJITA of our department in a symposium on the chromaffin, enterochromaffin and related cells held at Gifu, Japan, Aug. 22–24, 1975 (FUJITA, 1976). “Paraneuron” reminds us of the term “paraganglion” proposed by Kohn (1903). However, “paraneuron” indicates a cell, whereas “paraganglion” refers to an organ.

Paraneurons include adrenal and extra-adrenal chromaffin cells, gut endocrine cells, pancreatic islet cells, carotid body chief cells, parafollicular cells of the thyroid gland, parathyroid cells, bronchial and urogenital basal-granulated cells, gustatory cells of the taste bud, olfactory cells, rod and cone cells of the retina, hair cells of the inner ear, melanocytes and Merkel cells of the skin, and mast cells (FUJITA, 1976). Thus, paraneurons consist of a wider variety of cells as compared with the APUD-series proposed by Prof. A. G. E. PEARSE (1966, 1969, 1976).

According to the suggestion of Prof. A. YAMAUCHI (1976) of the Iwate Medical College, paraneurons are classified into the following three types: (1) Endocrine paraneurons (E type) including adrenal chromaffin cells, para-aortic or extra-adrenal chromaffin cells, gut endocrine cells, pancreatic islet cells, parafollicular cells of the thyroid gland, parathyroid cells, and adenhypophyseal cells. Their major function is endocrine, secreting amines and peptides as their products. (2) Internuncial paraneurons (I type) including small intensely fluorescent (SIF) cells or small granulated cells within or immediately adjacent to the sympathetic ganglia. There is increasing evidence that they are functionally interneurons intercalated between preganglionic sympathetic neurons and postganglionic neurons (WILLIAMS, 1967; WILLIAMS and PALAY, 1969; MATTHEWS and RAISMAN, 1969; WILLIAMS et al., 1976; MATTHEWS, 1976). (3) Sensory paraneurons (S type) including olfactory cells, gustatory cells in the taste buds, Merkel cells in the skin, hair cells of the inner ear and retinal rod and cone cells. They are all essentially chemo-, mechano-, or photo-receptors, respectively (FUJITA, 1976).

What type of paraneuron is the carotid body chief cell? To answer this question, the interpretation of the direction of innervations of the chief cell is important. If all the innervations of the chief cell are efferent as proposed classically by Kohn (1903) and WATZKA (1943) and recently by Biscoe and his co-workers (BISCOE, LALL and SAMPSON, 1970; BISCOE, 1971), it should be classified as an endocrine paraneuron. If the chemoreceptor is the nerve ending itself which makes a reciprocal synapse with the chief cell whose function is to modulate the sensitivity of the nerve ending against pO2 or pCO2 (McDONALD and MITCHEL, 1975a, b; MACDONALD, 1976), then the carotid body chief cell is an internuncial paraneuron. MACDONALD and MITCHEL (1975a, b) concluded that the carotid body chief cell is a dopaminergic interneuron. On the other hand, if the chief cell is a chemoreceptor receiving a sensory innervation as
proposed originally by DeCastro and supported by such electron microscopists as Kobayashi (1971b), Hess (1968, 1975), Verna (1971, 1973, 1975), Poulet-Krieger (1973), King and his co-workers (1975), Morgan, Pack and Howe (1975) and Osbourne and Butler (1975), this cell should be classified as a sensory paraneuron, although the models of the sensory synapses proposed by these electron microscopists are substantially different from author to author.

Materials and Methods

The experiment was carried out using male albino mice of dd-strain fed *ad lib*. The details of the animals used and the plan of experiment are shown in Table 1.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Body weight</th>
<th>Isotope</th>
<th>Dose of isotope</th>
<th>Method of injection</th>
<th>Time lapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20g</td>
<td>³H-leucine</td>
<td>200 μCi/gbw</td>
<td>Intraperitoneal</td>
<td>15 min</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>³H-leucine</td>
<td>100</td>
<td>³H-leucine</td>
<td>25 min</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>³H-leucine</td>
<td>100</td>
<td>³H-leucine</td>
<td>1.5 hr</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>³H-dopa</td>
<td>150</td>
<td>³H-dopa</td>
<td>15 min</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>³H-dopa</td>
<td>150</td>
<td>³H-dopa</td>
<td>30 min</td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>³H-dopa</td>
<td>50</td>
<td>³H-dopa</td>
<td>1 hr</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>³H-dopamine</td>
<td>20</td>
<td>³H-dopamine</td>
<td>30 min</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>³H-ATP</td>
<td>200</td>
<td>³H-ATP</td>
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<tr>
<td>9</td>
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<td>1 hr</td>
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<td>³H-ATP</td>
<td>100</td>
<td>Intravenous</td>
<td>1 hr</td>
</tr>
<tr>
<td>11</td>
<td>20</td>
<td>³H-ATP</td>
<td>100</td>
<td>Intraperitoneal</td>
<td>19 min</td>
</tr>
</tbody>
</table>

The following isotopes were purchased from the Radiochemical Center, Amersham, England:

1. ³H-leucine: L-(4, 5-³H) leucine; aqueous solution containing 2% ethanol; sp. act. 53 Ci/mmol.
2. ³H-dopa: L-3, 4-dihydroxy (ring-2, 5, 6-³H) phenylalanine; aqueous solution containing 2% ethanol; sp. act. 27 Ci/mmol.
3. ³H-dopamine: 3, 4-dihydroxy (ring-G-³H) phenylethylamine hydrochloride; aqueous solution containing 2% ethanol; sp. act. not lower than 2 Ci/mmol.
4. ³H-ATP: (2-³H) adenosine 5’-triphosphate, ammonium salt; aqueous solution containing 10% ethanol; sp. act. 16 Ci/mmol.

Soon after arrival, the isotopes were concentrated and dried under flowing nitrogen gas at room temperature, re-dissolved in distilled water, and applied to the animal in a final concentration of 10 μCi/μl.

Specimen preparation: The concentrated isotopes were injected either intraperitoneally or intravenously. After a range of predetermined times, the animals were anesthetized with ether gas and perfusion-fixed from the left ventricle of the heart with a 2.5% glutaraldehyde in a phosphate buffer of pH 7.2. Tissues at the carotid bifurcation containing the carotid body were removed and further fixed in the same glutaraldehyde fixative. Following an overnight fixation period, the tissue
 Autoradiography of Mouse Carotid Body  

pieces were post-fixed in 1.0% osmium tetroxide for 2 hr, dehydrated in a graded ethanol series and embedded in Luft’s Epon, using our standard procedure for the preparation of electron microscopic specimens.

Light microscope autoradiography: 1.0 μm sections cut on a Porter-Blum MT-1 microtome were placed on gelatin-coated glass slides. Autoradiograms were prepared by dipping the slides into liquid photographic emulsion (Ilford L4). Following an exposure of 4 days to 6 months, autoradiograms were developed in Kodak D-19, fixed in Fuji Fix, and stained through the emulsion with 0.5% toluidine blue in a phosphate buffer at pH 7.2 and 55°C for 12 to 15 sec, and then observed and photographed in the light microscope.

Electron microscope autoradiography: Specimens for electron microscope autoradiography were prepared from the same tissue blocks as those described above on the basis of the results of the light microscope autoradiography to estimate the optimum exposure time. Silver to gold sections cut with glass knives were transferred to collodion-coated glass slides using a thin sable brush, stained doubly with 0.5% uranyl acetate for 4 min and Millonig’s lead for 7 min, coated in vacuo with a thin layer of carbon-film and covered with a monolayer of Ilford L4 emulsion by the dipping method. After exposure of 23 days to 6 months, the emulsion was developed in Kodak D-19 for 3 min at 20°C and fixed in 30% sodium thiosulfate for 4 min and rinsed in 5 baths of distilled water for a total of about 5 min. Electron microscope autoradiograms were then transferred to a copper grid, dipped in isoamyl-acetate to remove the collodion-film and examined and photographed in Hitachi HU 125 DS electron microscope.

Results

1. Fine structure of the mouse carotid body
   The fine structure of the carotid body of various vertebrate species from amphibian to human has been extensively studied (KOBAYASHI, 1971b; BISCOE, 1971; KJAERGAARD, 1973; BÖCK, 1973; MCDONALD and MITCHEL, 1975a).
   The carotid body of the mouse is located on the superior cervical ganglion above the bifurcation of the common carotid into the internal and external carotid arteries (KOBAYASHI and UEHARA, 1970; KOBAYASHI, 1971b; BÖCK, 1973; BÖCK and GORGAS, 1976). The organ is organized into structural units called glomus complexes, chief cell units or chemoreceptor units (see KOBAYASHI, 1971b) around which there is a well-developed network of blood capillaries embedded in scanty connective tissue containing occasional mast cells (Fig. 1).
   The glomus complex consists of several chief or Type I cells which possess large dense-cored vesicles and small synaptic vesicles, sustentacular or Type II cells which ramify on and between the chief cells ensheathing the latter in a manner analogous to that of the satellite cells in the autonomic ganglion, and many nerve terminals closely associated with the chief cells and sustentacular cells.

2. Distribution of 3H-leucine-derived radioactivity
   L-leucine is a building block of peptide molecules. No autoradiographic work has previously been published on the uptake of radioactive leucine in the caroid body.
Fig. 1. An electron micrograph of the mouse carotid body illustrating at low magnification a glomus complex consisting of a chief cell (C), a sustentacular cell (S) and nerve terminals (n). Large dense-cored vesicles of the chief cell tend to accumulate along the cell membrane. \( \times 7,700 \)

Fig. 2. An electron micrograph showing the two types of membrane-bound particles in the chief cell. Large dense-cored vesicles (v) are dispersed throughout the cytoplasm, whereas small synaptic vesicles accumulate at the synaptic junction (arrows). An afferent nerve ending (A) with scattered non-cored synaptic vesicles is seen next to the chief cell. S sustentacular cell. \( \times 45,000 \)
Fig. 3. Result of autoradiographic grain-counting in the mouse carotid body after an intraperitoneal injection of $^3$H-leucine (No. 1-3). Method of grain-counting was as follows: (1) Grains on each structure were counted on the autoradiogram; (2) The weight of the photographic paper cut along the outline of each structure was measured in order to calculate the area occupied by it; (3) The radioactivity was expressed as a number of autoradiographic silver grains per unit area of a given structure. Symp. ggl. cell means the perikaryon of the nerve cell in the superior cervical ganglion. Non-myelin. nerve includes axons, Schwann cells and surrounding connective tissue in the non-myelinated nerve. Art. wall consists of endothelial cells, smooth muscle cells and elastic fibers. No. 1. 15 min after an intraperitoneal injection of 200 $\mu$Ci/gbw of $^3$H-leucine. 7-day-exposure. No. 2. 25 min after an intraperitoneal injection of 100 $\mu$Ci/gbw of $^3$H-leucine. 7-day-exposure. No. 3. 1.5 hr after an intraperitoneal injection of 100 $\mu$Ci/gbw of $^3$H-leucine. 9-day-exposure.

Fig. 4. A light microscope autoradiogram of the mouse carotid body, 15 min after the injection of $^3$H-leucine (200 $\mu$Ci/gbw; No. 1). Specific silver grains are scattered all over the cellular elements of the carotid body. $G$ indicates glomus complex and $v$, vascular lumen. 7-day-exposure. $\times 1,500$

Fig. 5. A light microscope autoradiogram showing nerve cells of the mouse superior cervical ganglion, 15 min after the injection of $^3$H-leucine (200 $\mu$Ci/gbw; No. 1). Cytoplasm of the nerve cells are much more heavily labeled than the nucleus. 7-day-exposure. $\times 1,500$
The present light microscope autoradiography showed that all kinds of cells in the carotid body and surrounding tissues take up $^3$H-leucine-derived radioactivity. At all time intervals (No. 1, 15 min; No. 2, 25 min; No. 3, 1.5 hr), the pattern of distribution of radioactivity observed as the autoradiographic grain density was similar. The highest radioactivity was seen in the perikaryon of the nerve cells of the superior cervical ganglion. In the nerve cells the cytoplasmic labeling was much higher than that of the nucleus. The glomus complex of the carotid body consisting of the chief cells, sustentacular cells and nerve terminals was less heavily labeled than the perikaryon of the nerve cell. In the glomus complex, distribution of autoradiographic silver grains was at random (Fig. 4).

Cells in the wall of the internal and external carotid arteries and in the non-myelinated nerves in and around the carotid body incorporated a considerable amount of $^3$H-leucine-derived radioactivity. Radioactivity in the mast cell was low. A few autoradiographic silver grains were seen on the fat cell, but no radioactivity was detected in the lipid droplet.

For a better survey, a grain counting was performed and the result is shown in Figure 3. Owing to the variation in the thickness of the sections and emulsion layers, in the difference in the dose of the isotope, in the exposure time of autoradiograms and in the photographic procedures, the counts indicate only roughly the order of the autoradiographic grain density in different sites.

![Fig. 6. An electron microscope autoradiogram showing a part of glomus complex containing chief cells (C), sustentacular cells (S) and nerve terminals (n), 15 min after the injection of $^3$H-leucine (200 µCi/gbw; No. 1). Silver grains are scattered on every cellular element of the glomus complex. Neither Golgi complex (g) nor peripheral portion of the chief cell is accompanied by accumulations of specific silver grains. 70-day-exposure. ×12,000](image)
In the present electron microscopic autoradiography, it was intended to clarify the distribution of ³H-leucine-derived radioactivity in the glomus complex, especially in the chief cell, at the level of cell organelles. Special attention was paid to whether an accumulation of radioactivity took place in the Golgi area or in the cytoplasmic area rich in the large dense-cored vesicles and small synaptic vesicles. However, no special accumulation of radioactivity in any part of the cell was recognizable at any time intervals after ³H-leucine injection (Fig. 6).

3. Distribution of ³H-dopa- and ³H-dopamine-derived radioactivity

³H-dopa: Distribution of radioactivity in the cells of the carotid body after injection of radioactive amines and amine precursors was previously studied by Gershon and Ross (1966) and Helpap and Hampel (1968) at a light microscopic level, and by Chen and Yates (1969) and Pouletter-Krieger (1973) at both light and electron microscopic levels. These authors have shown that the localization of exogenous amine precursors and their metabolites in the chief cell roughly coincided with that of the large dense-cored vesicles. Furthermore, Verna (1975) studied the distribution of ³H-noradrenaline-derived radioactivity in the rabbit carotid body. He reported the specific accumulation of autoradiographic silver grains on the nerve elements in the perivascular space and glomus complex. The chief cell did not seem to incorporate a significant amount of ³H-noradrenaline-derived radioactivity (Verna, 1975).

In the present study, specimens were examined 15 min (No. 4), 30 min (No. 5) and 1 hr (No. 6) after the ³H-dopa injection. No significant difference was found in the distribution of radioactivity among those three specimens. The heaviest radioactivity was seen in the cytoplasm of the mast cell in the connective tissue. The radioactivity in the mast cell expressed as the autoradiographic grain density was 5.5 to 25 times higher than that in the sympathetic ganglion cells (Fig. 7). The radioactivity in the glomus complex was also very high. It was frequently observed that the autoradiographic silver grains were concentrated along the periphery of the chief cell as shown in Figure 8. Radioactivity in the nucleus was always low.

Radioactivity in the sympathetic ganglion cells and non-myelinated nerves around the carotid body was fairly high. Practically no radioactivity was seen in the cells in the arterial wall, fat cells and connective tissue cells other than the mast cell.

In the electron microscope autoradiography, subcellular distribution of ³H-dopa-
derived radioactivity in the chief cell was examined. In all three specimens, the pattern of distribution of autoradiographic silver grains on the chief cell appeared to be similar. They showed no special association with mitochondria, Golgi complex or granular endoplasmic reticulum of the chief cell. Silver grains were scattered throughout the cytoplasm and in particular over the area rich in large dense-cored vesicles.

Incorporation of $^3$H-dopa-derived radioactivity in the nerve elements in the perivascular space was examined with special care. However, a striking accumulation of silver grains comparable to that seen in the specimen after $^3$H-dopamine injection (vide infra) was not detected.

$^3$H-dopamine: In the light microscope autoradiography of the carotid body 30 min after an intraperitoneal injection of $^3$H-dopamine (No. 7), striking accumulations of silver grains were seen in the connective tissue around the arteries of various thickness. Highly radioactive structures were also seen occasionally in the peripheral portion of the glomus complex (Fig. 11).

The distribution of the highly radioactive structures after $^3$H-dopamine injection apparently corresponded to that of adrenergic nerve terminals described by the
Fig. 10. An electron microscope autoradiogram of the mouse carotid body 15 min after the $^3$H-dopa injection (150 $\mu$Ci/gbw; No. 4). Distribution of specific silver grains roughly corresponds to that of the dense-cored vesicles in the cytoplasm of the chief cell. A few silver grains are also seen on the nucleus of the chief cell and other elements of the glomus complex. 27-day-exposure. $\times 12,000$

Fig. 11. A light microscope autoradiogram of the mouse carotid body 30 min after the intraperitoneal injection of $^3$H-dopamine (20 $\mu$Ci/gbw; No. 7). Striking accumulation of radioactivity is demonstrated on the nerve elements in the peri-arterial space and glomus complex. A few silver grains are also seen on the chief cell. 44-day-exposure. $\times 1,500$
previous authors (Biscoe and Stehbens, 1966; Böck, 1973; McDonald and Mitchell, 1975a, b; Kondo, 1971) which were mainly located in the perivascular space and less frequently next to the chief cell. Low but distinct radioactivity was also demonstrated in the cytoplasm of a restricted number of chief cells, whereas the majority of these cells exhibited no \(^3\)H-dopamine-derived radioactivity. This was confirmed by the electron microscopic autoradiography.

Practically no radioactivity was demonstrated in the sustentacular cell of the glomus complex, mast cell and other connective tissue cells and cells in the nerves in and around the carotid body.

4. Distribution of \(^3\)H-ATP-derived radioactivity

The uptake and intracellular distribution of \(^3\)H-ATP-derived radioactivity was studied in the hope to examine the possibility that the large dense-cored vesicles and/or small synaptic vesicles of the chief cell might incorporate exogenous ATP.

Specimens were obtained 15 min (No. 8), 1 hr (No. 9) and 19 hr after an intraperitoneal injection (No. 11) and 1 hr after an intravenous injection of \(^3\)H-ATP (No. 10). Typical examples of light and electron microscopic autoradiograms are illustrated in Figures 15 to 19. The result of light microscope autoradiographic grain
counting is shown in Figure 14.

In all the specimens examined the distribution of radioactivity was similar. Concentrations of radioactivity as judged from the density of autoradiographic silver grains were in this order: (1) glomus complex and mast cell, (2) sympathetic ganglion cell, non-myelinated nerve and tissue of the arterial wall, and (3) fat cell.

Careful examination of light microscopic autoradiograms revealed that, in all the kinds of cells investigated, radioactivity in the nucleus was conspicuously higher.

![Graph showing grain counts](image)

**Fig. 14.** Result of autoradiographic grain-counting in the mouse carotid body (No. 8-11) after $^3$H-ATP administration. Method used is the same as that for Figure 3. 
- **No. 8.** 15 min after an intraperitoneal injection of 200 µCi/gbw of $^3$H-ATP. 7-day-exposure.
- **No. 9.** 1 hr after an intraperitoneal injection of 200 µCi/gbw of $^3$H-ATP. 7-day-exposure.
- **No. 11.** 19 hr after an intraperitoneal injection of 100 µCi/gbw of $^3$H-ATP. 7-day-exposure.
- **No. 10.** 1 hr after an intravenous injection of 100 µCi/gbw of $^3$H-ATP. 7-day-exposure.

![Autoradiogram](image)

**Fig. 15.** A light microscope autoradiogram of the mouse carotid body, 1 hr after the intravenous injection of $^3$H-ATP (100 µCi/gbw; No. 10). Specific silver grains are seen on all kinds of cells in this picture. It is apparent that the nucleus incorporates more radioactivity than the cytoplasm. 7-day-exposure. $\times 1,500$
than that in the cytoplasm.

In the electron microscope autoradiography, subcellular distribution of radioactivity on the chief cell was examined. Autoradiographic silver grains associated with the large dense-cored vesicles, mitochondria, Golgi complex and endoplasmic reticulum were few, but most of them were located on the nucleus (Fig. 19).

Comparing the autoradiograms of the specimen taken 1 hr after an intravenous injection of 100 μCi/gbw of 3H-ATP (No. 10) with those of the specimen taken 1 hr after an intraperitoneal injection of 200 μCi/gbw of the same isotope (No. 9), it was apparent that the intravenous injection allows an incorporation of 3H-ATP-derived radioactivity several times higher than that obtained by the intraperitoneal injection (Fig. 14). No difference in the pattern of distribution of autoradiographic silver grains was noticed between the intraperitoneally-injected and intravenously-injected specimens.
1. Characteristic peptides in the chief cell

The presence of characteristic basophilic peptides in the large dense-cored vesicles of the carotid body chief cell was suggested by Capella and Solcia (1971) based on their examination of the reactivity of the membrane-bound particles of the normal human carotid body chief cell and chemodectoma cells to the reaction like masked metachromasia and lead-hematoxylin methods known to have a high specificity for basophilic peptides. Pearse who classified the carotid body chief cell into his APUD-series (Pearse, 1966, 1969, 1976; Pearse et al., 1973) proposed the name "glomin" for the provisional low-molecular peptide hormone of the chief cell (Pearse, 1969), though this substance has not yet been obtained even in a crude form.

Tramezzani, Morita and Chiocchio (1971) proposed that the carotid body is concerned with the production of erythropoietin. However, this proposal was not supported by Gillis and Mitchel (1973), Hansen and his co-workers (1973) and Paulo and his co-workers (1973).

It might be worthy of mention here that an immunohistochemical study in the author's laboratory using anti-ACTH-antisera, anti-somatostatin-antisera, anti-insulin-antisera, anti-glucagon-antisera, glucagon-like-immunoreactivity cross-reactive antisera and anti-neurotensin-antisera could not demonstrate any specific reaction in
the dog carotid body (Ito and Kobayashi, 1976: unpublished observation). Nevertheless, this negative result does not discourage at all the idea that the carotid body chief cells contain in their subcellular particles characteristic peptides such as “glomin” and/or the above-mentioned large molecule peptides with a high content of side chain carboxyl groups.

2. Monoamines in the chief cell and the two types of subcellular particles

Earlier arguments on the existence of monoamines in the carotid body chief cell have been resolved in previous studies (Kobayashi, 1971a; Böck, 1973; Böck and Gorgas, 1976). Occurrence of dopamine seems to be most probable. However, the kinds and nature of monoamines in the chief cell of the carotid body are still controversial among authors; species differences may account for some of the confusion (Rahn, 1961; Niemi and Ojara, 1964; Dearnaley, Fillenz and Woods, 1968a, b; Zapata et al., 1969; Knoche et al., 1969; Kobayashi, 1971a; Hervonen et al., 1972; Böck, 1973; Hellström and Koslow, 1976; Böck and Gorgas, 1976; Chen, Mascorro and Yates, 1976).

With regard to the subcellular localization of monoamines in the chief cell, it is still a matter of dispute whether they are located within the particles (see Fillenz, 1975).

Large dense-cored vesicles: It has been shown by the electron microscopic study that the large dense-cored vesicles incorporate various false transmitters such as 5-hydroxydopa (Hellström, 1971), 6-hydroxydopamine (Poullt-Krieger, 1973; Hess, 1975) and 5-hydroxydopamine (Poullt-Krieger, 1973; McDonald and Mitchel, 1975b). Furthermore, morphological changes in the large dense-cored vesicles in the animals given various pharmacologic agents such as reserpine were reported by Knoche, Decker and Schmitt (1971), Böck and Gorgas (1976) and Chen and his co-workers (Chen, Yates and Duncan, 1969; Chen, Mascaro and Yates, 1976). Although the results obtained were different in minor points from author to author, the opinions tend to the assumption that the main storing site of monoamines in the chief cell is the large dense-cored vesicle.

Small synaptic vesicles: Chemical composition of the small synaptic vesicles is unknown. Kobayashi and Uehara (1970) in the mouse and Kobayashi (1971b) in the guinea pig, Uroloncha domestica and Hynobius nigrescens reported that these vesicles contained granular precipitates. Vernia (1971, 1973) reported non-cored synaptic vesicles in the rabbit chief cell. McDonald (McDonald and Mitchel, 1975a, b; McDonald, 1976) claimed that the small synaptic vesicles only rarely contained dense cores. King and his co-workers (Hodges et al., 1975; King et al., 1975) reported that in the domestic fowl, some of the small synaptic vesicles were cored and the rest, non-cored. This discrepancy among authors concerning the ultrastructure of the small synaptic vesicles in the chief cell might be due to different glutaraldehyde fixatives used: Kobayashi and Uehara (1970) and Kobayashi (1971b) used phosphate buffered glutaraldehyde, Vernia (1971, 1973), cacodylate, McDonald (McDonald and Mitchel, 1975a, b; McDonald, 1976), hydrogen peroxide and King and his co-workers (Hodges et al., 1975; King et al., 1975), cacodylate buffered glutaraldehyde-formaldehyde mixture.

McDonald (McDonald and Mitchel, 1975b; McDonald, 1976) reported that in
the rat after treatment with 5-hydroxydopamine about 40% of small synaptic vesicles contained small dense precipitates. This is consistent with the idea that the small synaptic vesicles are not empty and might have a capacity to concentrate monoamines. McDonald (1976) also reported that, in the rat, a small fraction of small synaptic vesicles showed a peroxidase activity after an intraarterial infusion of horseradish peroxidase.

Further studies are needed to decide whether (1) the small synaptic vesicles in the chief cell contain monoamines; (2) whether they are involved in the storage and release of transmitter; (3) whether they play a role in the retrieval of the limiting membrane following the exocytotic release of the large dense-cored vesicles; or (4) whether the large dense-cored vesicles are precursors of the final small synaptic vesicles.

3. A summary of the results of autoradiography

The result of the present autoradiographic study on the uptake of $^3$H-leucine-, $^3$H-dopa-, $^3$H-dopamine-, and $^3$H-ATP-derived radioactivity in the mouse carotid body is summarized as follows:

(1) Carotid body chief cell incorporated less $^3$H-leucine-, more $^3$H-dopa- and slightly more $^3$H-ATP-derived radioactivity than the nerve cell of the superior cervical ganglion. Both kinds of cells did not incorporate $^3$H-dopamine-derived radioactivity in a large amount.

(2) As compared with another paraneuron, mast cell, the chief cell took up more $^3$H-leucine- and less $^3$H-dopa-derived radioactivity. Mast cells did not seem to use exogenous dopamine.

(3) $^3$H-ATP-derived radioactivity incorporated in the chief cell was fairly large in amount. The radioactivity was concentrated in the nucleus, whereas it was extremely low in the cytoplasm.

(4) Electron microscopic autoradiography after $^3$H-leucine injection suggested that newly synthesized radioactive peptides were dispersed both in the nucleus and cytoplasm and that no specific accumulation of them took place in the Golgi area at any time interval after the injection of the isotope.

(5) Distribution of $^3$H-dopa-derived radioactivity roughly coincided with that of the large dense-cored vesicles in the chief cell at all time intervals after the injection of the isotope. However, a few silver grains due to $^3$H-dopa were also seen on the nucleus and the cytoplasmic area lacking the dense-cored vesicles.

4. Movement of newly synthesized peptides and catecholamines

Using light and electron microscopic autoradiographic techniques, Coupland and Kobayashi (1976) examined cellular and subcellular distribution of recently synthesized peptides and catecholamines in the chromaffin cells of the mouse adrenal medulla after an intraperitoneal or intravenous injection of $^3$H-leucine and $^3$H-dopa, respectively. At 15 to 30 min after an intravenous injection of $^3$H-leucine, radioactive peptides were demonstrated mainly in the Golgi area, but at longer time intervals they were seen mostly in the granule-rich cytoplasm. At all time points after $^3$H-dopa injection the bulk of radioactivity which indicated the accumulation of newly synthesized catecholamines was present in the area of the cytoplasm rich in the mem-

Following the adrenal medulla, Kobayashi (1975, 1976) examined incorporation of \(^3\)H-leucine and \(^3\)H-dopa in the endocrine cells of the mouse stomach. The results of the experiment using \(^3\)H-leucine revealed that newly synthesized peptides were transported through the Golgi apparatus to the membrane-bound particles. An accumulation of radioactivity in the Golgi apparatus took place 15-30 min after the injection. On the other hand, the results of the \(^3\)H-dopa experiment supported the view that newly synthesized catecholamines are incorporated into the particles without passing through the granular endoplasmic reticulum and Golgi apparatus.

Based on the result of the experiments mentioned above Kobayashi (1976) suggested that one of the most important common characteristics in the chromaffin, enterochromaffin and related cells, which means paraneurons, is the uniform intracellular transport system for secretory peptides and amines. Thus, in the Golgi area, peptides are packaged into the membrane-bound particles, which then incorporate amines from the cytosol to become mature and are released from the cell surface by the process of exocytosis.

The result of the present study in the mouse carotid body, however, showed no accumulation of recently synthesized peptides in the Golgi area of the chief cell. This may be explained by assuming that the rate of secretion of peptides is low in the chief cell of the mouse carotid body. It may be possible that, even though the speculation is correct that the subcellular particles in the chief cell contain peptides and the latter are packaged into the particles in the Golgi area, the radioactivity incorporated into the newly-synthesized secretory peptides was so low, as compared with non-secretory peptides such as those in the nucleus, membrane and cytosol, that an accumulation of radioactivity in the Golgi area failed to become recognizable in autoradiography. This is consistent with the previous finding that definite signs of exocytosis were only rarely observed on the surface of the chief cell especially in the mouse carotid body (Böck and Gorgas, 1976; Chen, Mascarro and Yates, 1976).

The present study using \(^3\)H-dopa demonstrated the highest radioactivity in the cytoplasmatic area rich in the large dense-cored vesicles of the chief cell, confirming the results of the previous autoradiographic studies of Gershon and Ross (1966), Helpap and Kempel (1968), Chen and Yates (1969) and Poulet-Krieger (1973). No accumulation of radioactivity occurred in the Golgi area of the cell. Thus, the result of the present study encourages the view that, in the carotid body chief cell, as in the adrenal chromaffin cells and gut endocrine cells (Kobayashi, 1975, 1976; Coupland and Kobayashi, 1976; Coupland, Kobayashi and Crowe, 1976; Coupland, Kobayashi and Kent, 1976), catecholamines are incorporated in the dense-cored vesicles without passing through the Golgi area.

5. Evaluation of \(^3\)H-ATP autoradiography

It has been proposed that the stimulation of the carotid body chemoreceptor results from a failure to form ATP at a rate to keep pace with its utilization in the chemoreceptor (Anichkov and BeLEN'KII, 1963; Joels and Neil, 1968). However, the purpose of the present autoradiographic study using \(^3\)H-ATP was not to elucidate
the significance of ATP in the metabolic activity of the cell but to clarify whether exogenous ATP might be used in combination with peptides and monoamines, for the formation of a storage complex in the membrane-bound particles of the chief cell.

Since there are lots of cytochemically detectable phosphatases in the cell membrane around the chief cell (Prys-Davies, Dawson and Westbury, 1964; Palkama, 1965; Becker, Drukker and Meijer, 1967; Fine, Enriquez and Morales, 1968), exogenous ATP may be broken down before crossing the cell membrane of the chief cell. However, it may be possible that ATP penetrated through the cell membrane in the form of AMP or other ATP-derivatives is rapidly resynthesized within the chief cell (see Tornnace, 1968, p. 111).

Anyhow, the result of the present study showed that $^3$H-ATP-derived radioactivity does enter the chief cell, and that the radioactivity was gathered in the nucleus of the cell. A parallel study in the adrenal chromaffin cells and gut endocrine cells showed that these cells also incorporate $^3$H-ATP-derived radioactivity (Kobayashi, unpublished). In the gastric fundus the incorporation of $^3$H-ATP-derived radioactivity in the endocrine cells looked almost specific to them, whereas neither parietal nor chief cells of the fundic gland appeared to incorporate $^3$H-ATP in a large amount (Kobayashi, unpublished). In both adrenal chromaffin cells and gut endocrine cells, autoradiographic silver grains due to $^3$H-ATP were distributed throughout the cell so that the grain density did not differ between the nucleus and the cytoplasm.

It is not known why, in the carotid body, $^3$H-ATP-derived radioactivity was far higher in the nucleus than in the cytoplasm, while nuclear and cytoplasmic radioactivity was nearly equal in the adrenal chromaffin cells and gut endocrine cells. However, one of the possible explanations might be that both adrenal chromaffin cells and gut endocrine cells are active in the formation of secretory products so that they need large amounts of ATP in the cytoplasm as well as in the nucleus, while the carotid body chief cell is not so active in the production of secretory materials resulting in the limited use of exogenous ATP in the cytoplasm.

マウスの頸動脈小体に関する $^3$H-ロイシン, $^3$H-ドバ, $^3$H-ドバミン および $^3$H-ATP をもちいたオートラジオグラフィー：とくに

バラニューロンとしての主細胞について

小 林

主細胞の バラニューロンとしての特性を 検討する目的で、マウス頸動脈小体での 三重水素 (3H) で標識したロイシン、ドバ、ドバミンおよびATPの、各種細胞への取込みを 光顕および電顕オートラジオグラフィーを手段として 研究した。

新生された蛋白質の存在を示す$^3$H-ロイシン由来の放射能は、頸動脈小体とその周囲組織のすべての種類の細胞に認められた。主細胞の放射能は、上頸神経節細胞のそれより すべての時点で低かった。電顕オートラジオグラフィーでは、主細胞において、特殊粒粒の 新生がおこなわれるとと思われるゴルジ野にも、また成熟した特殊粒粒が貯蔵されると予想
される細胞質周辺部にも、$^3$H-ロイシン由来の放射能の蓄積は認められなかった。
新生されたカテコールアミンの存在を示す $^3$H-ドパ由来の放射能は、主細胞と上頸神経節細胞と肥満細胞とに、特異的に認められた。主細胞での放射能の分布は、大型芯あり、小胞の分布とほぼ一致していた。

$^3$H-ドパミン注射後には、主として動脈周囲の神経終末に、大量の放射能の取込みが認められた。また、主細胞と支持細胞に接して同様の神経終末がみられたことがある。一部の主細胞は、$^3$H-ドパミン由来の放射能を取込むが、残りのものには取込みが認められなかった。

$^3$H-ATP に由来する放射能は、頸動脈小体とその周囲組織中のすべての細胞に見出された。主細胞では、他の細胞においても同様、核の放射能がもっとも強くあった。

頸動脈小体主細胞は、他のパラニューロンと同様に、その大型芯あり小胞または小型シナプス小胞の中に、分裂形または興奮伝達物質として、ペプチドと活性アミンと ATP の三者を同時に貯蔵していると仮定されるが、今回の実験より、これらの産生および放出の速度は、クロム親和細胞や胃腸内分泌細胞に比べると非常に遅いものと予想される。

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