periments do not elucidate the cause of the anaphylactic electrocardiographic changes. However, many observations suggest that coronary vasoconstriction is the main cause of myocardial reaction in anaphylactic shock although anoxemia due to constriction of pulmonary arterial vessels and bronchospasms may also play an important role in this connection[1-8, 11]. A direct anaphylactic reaction of the myocardial tissue has also to be taken into consideration since arrhythmias have been observed in anaphylaxis in isolated papillary muscle[10].

Although blood pressure eventually decreases because of diminished venous blood return resulting from peripheral vasoconstriction which was demonstrated by many investigators[1, 9], it seems most likely that diminished cardiac work also contributes to the blood pressure decrease occurring in experimental and clinical anaphylactic shock. This point of view is supported by observations made in isolated guinea-pig hearts in anaphylaxis giving evidence of strong coronary vasoconstriction followed by a significantly decreased cardiac output[5, 6].

Under the aspect of the electrocardiographic changes observed by us and others[5, 7, 8, 11], an undifferentiated therapy of anaphylactic shock with epinephrine and similar substances should be reconsidered despite the fact that it may decrease pulmonary resistance[3] and overcome peripheral vasoconstriction[1, 9]. Epinephrine as well as isoprenaline may eventually increase arterial blood pressure and peripheral tissue perfusion, but they may also induce ventricular extrasystolias by themselves and/or increase myocardial oxygen deficiency as detectable in the electrocardiogram[12]. It is also questionable whether the use of pressoric substances such as norepinephrine or angiotensin can be of value in a situation in which maximal vasoconstriction already prevails[9, 12].

Zusammenfassung. Die simultane Registrierung des EKG und des Karotisblutdrucks am Kaninchen in der Frühphase des anaphylaktischen Schocks lässt auf eine primäre Herzreaktion schliessen, welche nicht auf einer Verminderung des Blutdrucks, bzw. des koronaren Perfusionsdrucks, sondern auf einer vorwiegend infolge Koronarkonstriktion eintretenden Abnahme der Herzleistung zu beruhen scheint.

A. WEGMANN, H. RENKER and H. HÜBSCHER

Swiss Serum and Vaccine Institute, Rehhagstrasse 79, CH-3001 Bern (Switzerland), 4 January 1972.

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Amine and Amino Acid Microanalysis of Two Identified Snail neurons with Known Characteristics

Because of the known heterogeneity of neurons it would seem that precise biochemical information on the functioning of nervous systems is best obtained from experiments on individual neurons. Certainly we will need to learn more of the cellular biochemistry occurring in neurons during adaptive behavioural activity before fully comprehending the underlying processes controlling these events.

In the present work we have used a recently devised micromethod[1] to analyse the amine and amino acid composition of two specified giant neurons in the snail (*Helix pomatia*) brain. The method involves the reaction of amines and amino acids with ^{14}C-labelled dansyl chloride to form compounds which can be separated by thin layer chromatography and detected under UV-light or by autoradiography. One of the neurons studied, the giant metacerebral, or giant serotonin cell (GSC)[2] receives both excitatory (cholinergic)[3] and inhibitory innervation. The inhibitory effect is mimicked by glutamic acid[4]. There are two identical GSCs in each snail brain; one is located in each cerebral ganglion. Evidence that these cells contain serotonin comes from fluorescence histochemical and bioassay data[2]. The other cell analysed, the so called posterior buccal cell receives an excitatory (serotonergic) input from each GSC[5, 6]. This cell lacks the capacity, shown by the GSC, to form serotonin from 5-hydroxytryptophan[7] and histochemical studies suggest that the cell lacks serotonin. There is one posterior buccal cell in each buccal ganglion.

The main objectives of our study were as follows: 1. To determine whether it is possible to obtain consitent data when analysing a small number of GSCs or posterior buccal cells dissected from different animals. 2. To obtain biochemical data on the content of serotonin in both types of neurons. 3. To establish whether there are any differences in the amino acid composition of the two types of cells. 4. To obtain further data on the effect of optic tentacle ablation on the level of serotonin in the GSC, since previous results suggested that this procedure causes a reduction in serotonin[8].

The identified neurons were dissected by hand from ganglia removed from live snails. The cells were transferred to a Drummond Microcap containing 3 µl of cold 0.05 N $NaHCO_3$ (adjusted to pH 9.5 with 1 N NaOH). When sufficient cells were collected they were homogenised, 3 µl of acetone was added and the mixture cooled to −5°C for 1 h. After centrifuging at 4,000 g for 30 min the supernatant was mixed with 4 cl of a 2 mg/ml solution ^{14}C labelled dansyl chloride ((1 dimethyl ^{14}C) aminonaphthalene-5-sulphonyl chloride)[9] in acetone. Each sample was incubated at 37°C for 1 h and spotted in the corner of 3 × 3 cm polyamide layer chromatography sheet (Carl, Schneider and Schüll, F 170 Mikropolyamide). Chromatograms were developed in water/formic acid (100:3) in one dimension and in benzene/acetic acid (9:1) in the second dimension, and viewed in UV-light. Autoradiograms were prepared using Afga-Gevaert Denture Ultra Rapid L film.

Best results were obtained with extracts from 8 cells although satisfactory chromatograms were also obtained when 4 cells were used. Examination of 16 pairs of chromatograms, each prepared from 8 cells showed that it is indeed possible to obtain consistent results for each cell type. Figure 1 shows the autoradiograms of 3 pairs of chromatograms, those of the posterior buccal cells above,

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[4] G. A. GOTTRELL, J. MACON and A. C. SZCZEPANIAK, in preparation (1972).
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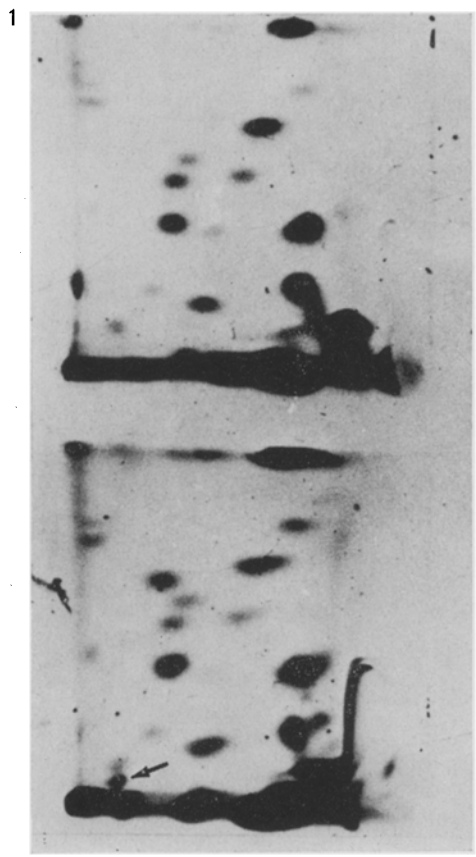
[6] G. A. GOTTRELL, Experientia *27*, 813 (1971).
[7] G. A. GOTTRELL and B. POWELL, J. Neurochem. *18*, 1695 (1971).
[8] N. N. OSBORNE and G. A. COTTRELL, in preparation (1972).
[9] Centre d'Etudes Nucléaires de Saely, Gif-sur-Yvette, France, specific activity 49 mC/mMole.



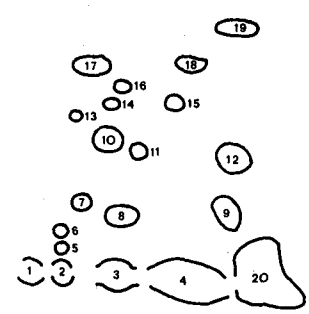


Fig. 1. Autoradiograms of 3 pairs of microchromatograms and an identification key. The upper chromatogram in each pair was prepared from the posterior buccal cell and the one below from the GSC. Pairs 1 and 2 contained material from normal animals. Chromatography was completed the day of dissection in the case of 1, but one day after dissection for 2. The 3rd pair of chromatograms contains cells of animals from which the optic tentacles had been removed 6 h before dissection for analysis. A spot corresponding to the position of serotonin (arrow in 1) is seen in each GSC chromatogram, but not in any of those of the posterior buccal cell. A faint spot corresponding to the position of methionine is seen in every chromatogram of the buccal cell. This spot is very much less intense, if present at all in the lower GSC chromatograms. The intensity of spot 17, an unidentified compound, varies greatly from one chromatogram to another. The other pronounced spots were identified as follows: 1 starting point, 2 unknown A, 3 taurine, 4 dansyl-OH, 5 N-serotonin, 6 tryptophan, 7 *bis*-lysine, 8 bis-ornithine, 9 glycine, 10 phenylalanine, 11 methionine, 12 alanine plus dansyl-HN_2, 13 unknown B, 14 leucine, 5 valine, 16 isoleucine, 17 unknown C, 18 proline, 19 unknown D, 20 glutamine, threonine, asparagine, serine, argenine, ε-lysine, α-amino-histidine, cystine, aspartic acid, glutamic acid. Spots corresponding to the position of ethanolamine (to the right and above spot 12) and *bis*-tyrosine (to the left of spot 13) were also occasionally seen.

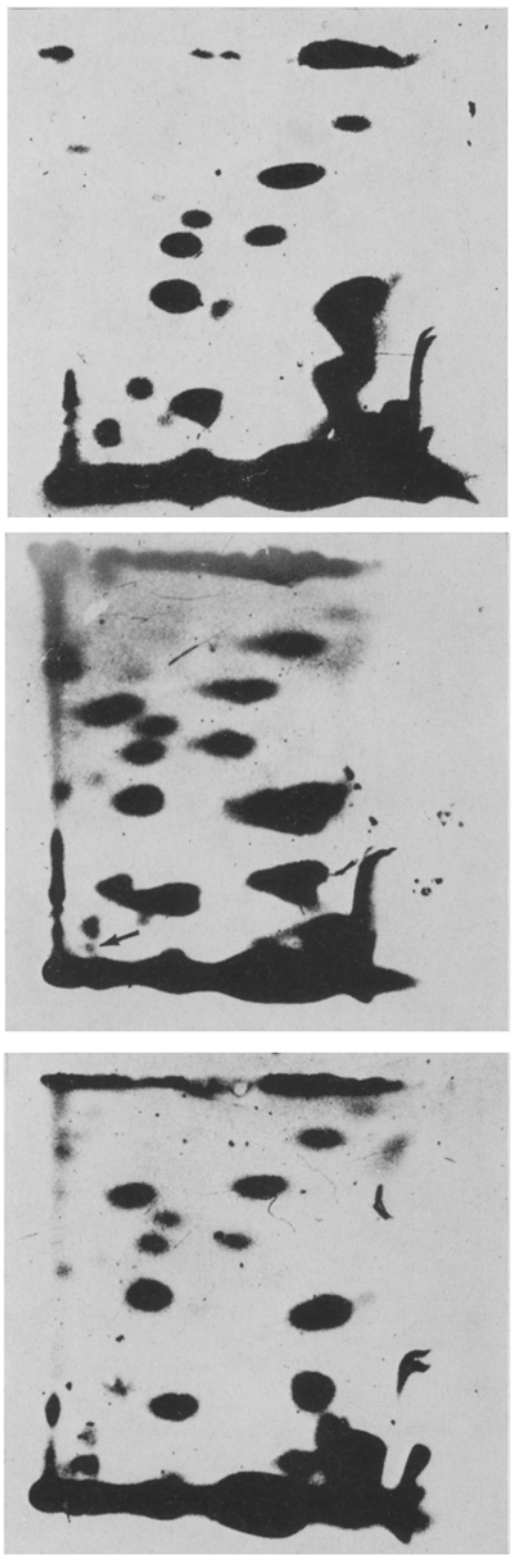


Fig. 2. The effect of optic tentacle ablation 8 days prior to analysis on the serotonin level and amino acid composition of the GSC and posterior buccal cell. The intensity of the serotonin spot (arrow) in the middle GSC chromatogram, prepared from the operated animals, is much reduced, especially in relation to the other spots, compared with the situation in the control GSC chromatogram (bottom chromatogram prepared from normal animals) and also with the three GSC chromatograms shown in Figure 1. As before the level of methionine is higher in the buccal cell chromatogram, the one at the top, than in the GSC.

and the GSC beneath, in each case. A spot corresponding to the position of serotonin (at the pH of the reaction serotonin appears almost completely as an N-derivative) can be seen in each GSC (arrow in number 1) chromatogram, but not in the chromatograms of the buccal cells, although in the buccal cell chromatograms the spot immediately above the position of serotonin, i.e. tryptophan, is clearly seen. If GSC extracts were stored for 3 to 14 days before dansylation the intensity of the serotonin spot was reduced, or the spot lost. Serotonin is known to be labile at an alkaline pH. The overall pattern of the other substances was similar for both cell extracts. However, one difference was the level of methionine which was consistently greater in the posterior buccal cell. Furthermore, an unidentified substance, spot 17, varied greatly in intensity in both cells. In some cases, it could barely be detected (see e.g. chromatogram 1 of the buccal cell and 3 of the GSC) whereas in other chromatograms its presence was obvious (chromatogram 2 of the buccal cell and 2 of the GSC). Sometimes there was a slight variation in the intensity of certain spots with respect to other ones in different chromatograms (e.g. spot 18 appears more intense than spot 19 in chromatogram 1 GSC, whereas the situation is reversed in chromatogram 2 GSC).

The level of serotonin was not noticeably changed in the GSC when optic tentacles were removed 8 h before analysis (chromatogram 3), but 8 days after tentacle ablation the serotonin level was definitely reduced (Figure 2).

We can therefore conclude that it is possible to obtain consistent results on the serotonin and amino acid content of the 2 different neurons. The experiments provide conclusive proof of the presence of serotonin in the GSC, whereas the level of this amine in the posterior buccal cell is very much lower, if present at all. 8 days after optic tentacle ablation there is a pronounced reduction in the serotonin level in the GSC. The reason for this effect is at present unclear; the GSCs do not appear to send axons to the tentacles, but they receive complex synaptic inputs from the tentacle nerves[7].

Another unexplained result is the pronounced fluctuation in the concentration of the unknown substance, spot 17. This substance was also detected in homogenates of the snail brain and its level is increased by electrical stimulation of the brain[10]. Further studies are required to identify this interesting compound and to analyse its role in neurons. The observation that the level of methionine is consistently higher in the buccal cell also merits further investigation.

Résumé. La microanalyse de l'amine et de l'amine acide de deux neurones identifiés a prouvé la présence de sérotonine dans l'un des neurones et son absence dans l'autre, celui qui contenait plus de méthionine. A l'exception d'un intéressant composé non identifié, les autres substances étaient essentiellement semblables dans les deux neurones.

N. N. Osbone[11] and G. A. Cottrell

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[11] Present address: Arbeitsgruppe Neurochemie, Max-Planck-Institut für Experimentelle Medizin, 3400 Göttingen (Germany).