cation au lindane dans le système nerveux central de *Musca domestica*[1] et les corpora cardiaca de *Locusta*[2], nous remarquons une modification des grains de neurosécrétion. Chez les femelles adultes normales, les 4 types de fibres neurosécrétrices décrits par Cassier et Fain-Maurel[10] renferment de nombreux grains de nature soit peptidique soit monoaminergique[4]. Au cours du cycle ovarien, ces grains sont denses dans leur immense majorité alors que dans les corpora allata des femelles inactives ou en fin de cycle, le contenu des grains deviendrait transparent[11].

Quel que soit le type de fibres considéré, nous remarquons aussi bien chez les larves du 5e stade que chez les femelles aux corpora allata actifs intoxiquées au lindane de profondes modifications. De façon générale, on constate une altération des grains. Les parois de certains d'entre eux paraissent se dissocier de sorte qu'ils se vident dans l'axoplasme environnant. En outre, la densité des grains décroit beaucoup lors de l'intoxication et les fibres de type IV ne renferment pratiquement que des grains «vides» même chez les femelles en cours de premier cycle ovarien (figure 4).

Discussion. Les modifications qui nous paraissent les plus importantes, quant à leur signification physiologique, parmi nos diverses observations, sont celles qui concernent le reticulum endoplasmique lisse et celles des terminaisons neurosécrétrices. En revanche, les altérations lysosomiales, mitochondriales et vacuolaires sont certainement non spécifiques car elles ont été décrites dans d'autres organes après action de diverses substances toxiques. Nos résultats sont à rapprocher de travaux récents relatifs au mode d'action du précocène chez le même insecte[12]. Schooneveld étudiant les effets du précocène, un nouvel insecticide provoquant la mue imaginale de façon anticipée, observe des lésions ultrastructurales chez *L. migratoria* au cours du 4e stade larvaire. En particulier, il observe une dilatation des espaces intercellulaires dans les corpora allata des insectes intoxiqués après 90 min d'intoxication qu'il met en rapport avec un arrêt d'activité de la glande. Nos propres recherches nous ont également conduit à mettre en évidence de telles altérations des espaces intercellulaires après intoxication par le lindane. Les modifications que nous avons décrites dans le reticulum endoplasmique dans les cellules glandulaires réputées actives au moment de l'intoxication par cet insecticide et les altérations ultrastructurales observées au niveau des feuillets suggèrent que le lindane, lors de l'intoxication aiguë provoquerait une inhibition de l'activité glandulaire.

1 F. Ramade, Annls Inst. nat. Agron. Paris *5*, 1 (1967).
2 B. Moreteau et F. Ramade, Zool. Jb. Physiol. *83*, 340 (1979).
3 R. Pasquier, Bull. Off. nat. anti-acridien Alger *2*, 5 (1947).
4 P. Cassier, Int. Rev. Cytol. *57*, 1 (1979).
5 L. Joly, A. Porte et A. Girardie, C. r. Acad. Sci. Paris *265*, 1633 (1967).
6 L. Joly, P. Joly, A. Porte et A. Girardie, Archs Zool. exp. gén. *109*, 703 (1968).
7 L. Joly, P. Joly, A. Porte et A. Girardie, Archs Zool. exp. gén. *110*, 617 (1969).
8 M.A. Fain-Maurel et P. Cassier, Z. Zellforsch. mikrosk. Anat. *102*, 543 (1969).
9 M.A. Fain-Maurel et P. Cassier, C. r. Acad. Sci. Paris *268*, 2721 (1969).
10 P. Cassier et M.A. Fain-Maurel, Z. Zellforsch. mikrosk. Anat. *111*, 483 (1970).
11 M. Guelin et A. Darjo, C. r. Acad. Sci. Paris *278*, 491 (1974).
12 H. Schooneveld, Experientia *35*, 363 (1979).

Sodium and calcium action potentials in human anterior pituitary cells

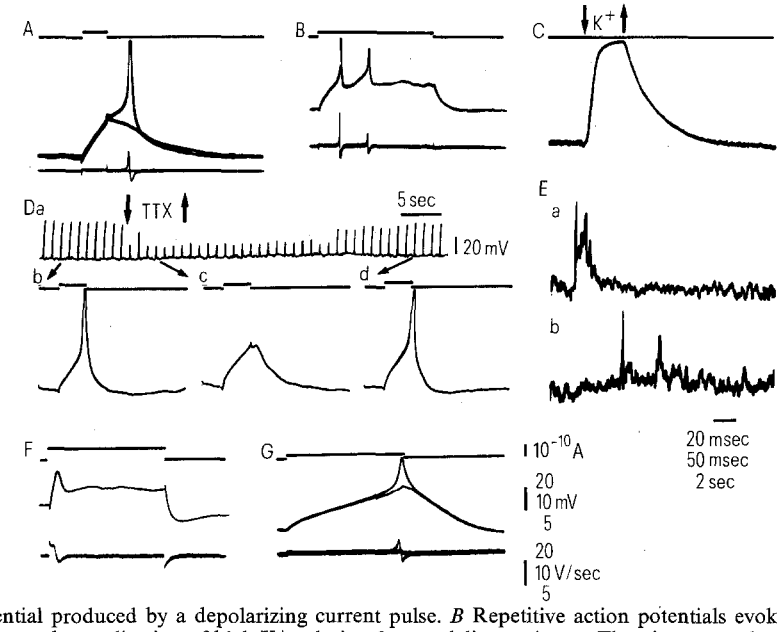
S. Ozawa and T. Saito[1]

Department of Physiology and Endocrinology and Metabolism, Jichi Medical School, Tochigi-ken 329-04 (Japan), 10 December 1979

Summary. Human anterior pituitary cells derived from a somatotropin-secreting adenoma were capable of generating action potentials with Ca^{2+} and tetrodotoxin-sensitive Na^+ components. A major fraction of the action current was carried by Na ions.

Both normal and neoplastic anterior pituitary cells derived from rat and fish are capable of generating action potentials due to an increase in the membrane permeability to Ca and Na ions[2-4]. Because release of secretory products is initiated by Ca influx in most secretory cells, the Ca component of the action potential has been suggested to play a role in the control of secretion of anterior pituitary hormones[5-8]. Biales et al.[2] have shown that human anterior pituitary cells also generate action potentials with a Na component. However, the detailed ionic mechanism of the action potential remains to be determined. This paper reports that action potentials in human pituitary cells have both Ca and tetrodotoxin-sensitive Na components and that the latter is dominant for spike initiaion.

Material and methods. Human pituitary cells were obtained from an adenoma removed by transsphenoidal surgery in a 64-year-old woman who had developed typical symptoms of acromegaly during the last 16 months. The resting level of growth hormone in plasma determined by radioimmunoassay ranged from 26.4 to 106 ng/ml. This value is 10–50 times higher than in normal adults. Electron microscopical survey revealed that the entire cytoplasma of the cells in the adenoma tissue was filled with round dense secretory granules. The diameter of the granules was 344 ± 6 nm (mean $\pm$ SEM $n = 77$). The cell suspension was prepared according to the method of Vale et al.[9]. The cells were grown in 35×10 mm Falcon dishes containing 2 ml of Ham's F-10 medium supplemented with 15% horse serum and 2.5% fetal bovine serum. The experiments were conducted 1–7 days after plating. During this period the cells were round-shaped and attached to the bottom of the dish. Intracellular recording and current injection using a bridge circuit were done as described previously[10] in a standard solution of the following composition (mM): NaCl 138, KCl 5, $CaCl_2$ 10, $MgCl_2$ 1.3, glucose 10 and either Hepes-NaOH or Tris-HCl buffer 5 at pH 7.4. In some experiments the Ca^{2+} concentration was reduced to 2.4 mM by increasing Na^+ on an isotonic basis. Na-free solution was prepared by substitution of choline for Na^+. Tetrodotoxin (TTX) was applied by pressure ejection from a 5-µm-tip micropipette filled with recording medium containing 15 µM TTX. The tip of the pipette was transiently located



A All-or-none action potential produced by a depolarizing current pulse. *B* Repetitive action potentials evoked by a prolonged current pulse. *C* Depolarizing response by application of high K^+ solution from a delivery pipette. The pipette was located about 30 µm from the cell at the downward arrow and moved away at the upward arrow. The spike generating mechanism was injured in this cell. *D* Effect of tetrodotoxin (TTX). Action potentials were evoked by depolarizing current pulses. *a* Pen recorder trace showing the whole time course of the effect of TTX. *b, c, d* Oscilloscope traces of the parts indicated by arrows in record *a.* This recording was done in normal saline containing 2.4 mM Ca^{2+}. *E* Small potential fluctuations and action potentials which occurred spontaneously. The peaks of the 2 action potentials are truncated in this recording. *a* and *b* are 2 successive traces. *F* Graded response by a depolarizing current pulse in Na-free solution containing 2.4 mM Ca^{2+}. *G* All-or-none action potential in Na-free solution containing 24 mM Ca^{2+}.
The base line of the current trace indicates the 0 mV level of the potential trace. In A, B, F and G the bottom traces represent the first order derivative of the potential trace. The voltage calibration of 20 mV applies to B, F, and G, that of 10 mV to A, C, Db-Dd,, and that of 5 mV only to E. The calibration of 20 V/sec applies to A, that of 10 V/sec to B, and 5 V/sec to F and G. The time calibration of 20 msec applies to A, Db-Dd, that of 50 msec to B, F and G, and that of 2 sec to C and E.

about 30 µm from the penetrated cell. When 150 mM K^+ solution was ejected from a similar pipette in this position, the membrane potential depolarized by more than 40 mV as shown in the figure, C. All experiments were carried out at 31–36 °C.

Results and discussion. The resting potential in these pituitary cells in the standard solution was 41.0±4.1 mV (mean ± SEM, n=16). The input resistance estimated by applying hyperpolarizing current pulses was 398±37 MΩ (n=5). Action potentials could be evoked either at the termination of a hyperpolarizing current pulse or during a depolarizing pulse. The maximum rate of rise of the all-or-none action potential generated by a brief depolarizing current pulse as shown in the figure, A, was 18.3±1.9 V/sec (n=9). A prolonged current pulse of suprathreshold intensity elicited repetitive action potentials (figure, B). Spontaneous action potentials occasionally occurred. In such cases small potential fluctuations were seen to occur randomly, and action potentials might be initiated from these potentials (figure, E).

In order to understand the ionic mechanism of the action potential, TTX was applied to the penetrated cells from a delivery pipette. Application of TTX immediately caused the major fraction of the action potential evoked by a depolarizing current pulse to disappear (figure, D). The effect of TTX was reversible, and 20–30 sec after removal of the delivery pipette the original shape of the action potential was restored (figure, D b, c and d). In Na-free solution the all-or-none action potential was also abolished. Action potentials in these cells are therefore mainly due to an increase in the membrane permeability to Na^+. However, significant graded depolarizing responses were always observed in Na-free solution containing 2.4 mM Ca^{2+} as well as in the presence of TTX (figure, F). When the Ca^{2+} concentration of the Na-free solution was increased to 24 mM, all-or-none action potentials could be generated by passing depolarizing current pulses (figure, G). The maximum rate of rise of these action potentials was 2.4±0.4 V/sec (n=6). Since the corresponding value in the standard saline containing 10 mM Ca^{2+} was 18.3 V/sec as described above, only a minor fraction of the action current is carried by Ca^{2+} in these cells. In this respect the human anterior pituitary cells are quite similar to the normal anterior pituitary cells of rat (type I)[3] and fish[4]. The functional significance of the Na component of the action potential remains to be elucidated.

1 We wish to thank Dr O. Sand for correcting the English and Miss Michiko Takano for technical assistance. We also acknowledge the collaboration of Dr S. Miyazaki in the electron microscope.
2 B. Biales, M.A. Dichter and A. Tischler, Nature *267*, 172 (1977).
3 S. Ozawa and O. Sand, Acta physiol. scand. *102*, 330 (1978).
4 P.S. Taraskevich and W.W. Douglas, Nature *276*, 832 (1978).
5 Y. Kidokoro, Nature *258*, 741 (1975).
6 P.S. Taraskevich and W.W. Douglas, Proc. natl Acad. Aci. USA *74*, 4064 (1977).
7 B. Dufy, J.D. Vincent, H. Fleury, P. Pasquier, D. Gourdji and A. Tixier-Vidal, Science *204*, 509 (1979).
8 S. Ozawa and N. Kimura, Proc. natl Acad. Sci. USA, in press.
9 W. Vale and G. Grant, in: Methods in Enzymology, vol.37, part B, p.82. Ed. B.W. O'Malley and T.G. Hardman. Academic Press, New York 1975.
10 S. Ozawa and M. Miyazaki, Jap. J. Physiol. *29*, 411 (1979).