I_{Na} was largely blocked during high frequency stimulation with bradykinin diffusing into the axoplasm from the two ends of the fiber. In many respects the effects of internally applied bradykinin resemble those of leu- and met-enkephalins[16] and those of quaternary derivatives of the local anesthetics lidocaine and trimecaine[17,18]. One interpretation of the internal bradykinin effect is that during depolarizations, the molecules would bind to a site located in the neighborhood of the sodium channel and then immobilize the sodium channel gate (related to inactivation of the sodium permeability) leaving the channel in a closed configuration. The fast sodium inactivation process can be eliminated by internal treatments with proteolytic enzymes[19], which suggests that the 'h' gate is composed of a polypeptidic chain. It is tempting to postulate that bradykinin molecules enter the sodium channels from their axoplasmic end and bind to the receptors for the 'h' gate. The results of Eaton et al.[20] make the amino acid arginine a likely component of the sodium inactivation gate; bradykinin has two arginine residues which could bind to the receptor for the 'h' gate. However, since enkephalins have no arginine residue, we postulate that enkephalins would bind to the receptor for the 'h' gate by another amino acid, the most serious candidate being phenylalanine[19]. Bradykinin has phenylalanine residue too; therefore, more than one residue may be involved in the interaction between bradykinin molecules and sodium channels; the positively charged residues could interact with a site located at or near the extracellular surface of sodium channels whereas the neutral residues could react with a site within the membrane and on the cytoplasmic surface.

The slowing of I_{Na} inactivation and the increase of late current could account for the exciting activity of bradykinin, which can form aggregates, producing polypeptidic neurotoxins acting as 'modifiers' of the sodium channel gate and promoting excitation of axons.

Acknowledgments. This study was supported by a grant from the Italian National Research Council (CNR No. CT 860097).

1 Bergman, C., Dubois, J. M., Rojas, E., and Rathmayer, W., Biochim. biophys. Acta *455* (1976) 173.
2 Catterall, W. A., A. Rev. Pharmac. Tox. *20* (1980) 15.
3 Neumcke, B., Schwarz, W., and Stämpfli, R., Biochim. biophys. Acta *814* (1985) 111.
4 Beck, P. W., and Handwerker, H. O., Pflügers Arch. *347* (1974) 209.
5 Lewis, G. P., and Reit, E., J. Physiol. *179* (1965) 538.
6 Juan, H., and Lembeck, F., Archs Pharmac. *283* (1974) 151.
7 Szolcsanyi, J., J. Physiol. *338* (1987) 9.
8 Campese, V. M., and Mitolo-Chieppa, D., in: Vasopeptides. Chemistry, Pharmacology and Pathophysiology, p. 477. Eds N. Back and F. Sicuteri. Plenum Publishing Corp., New York 1971.
9 Nonner, W., Pflügers Arch. *309* (1969) 176.
10 Dubois, J. M., and Bergman, C., Pflügers Arch. *370* (1977) 185.
11 Chiu, S. Y., J. Physiol. *273* (1977) 573.
12 Dubois, J. M., and Bergman, C., Pflügers Arch. *357* (1975) 145.
13 Meves, H., Prog. Biophys. molec. Biol. *33* (1978) 207.
14 Frankenhauser, B., and Hodgkin, A. L., J. Physiol. *137* (1957) 218.
15 Hille, B., J. gen. Physiol. *51* (1968) 221.
16 Carratù, M. R., Dubois, J. M., and Mitolo-Chieppa, D., Neuropharmacology *21* (1982) 619.
17 Khodorov, B., Shishkova, L., Peganov, E., and Revenko, S., Biochim. biophys. Acta *433* (1976) 409.
18 Strichartz, G. R., J. gen. Physiol. *62* (1973) 37.
19 Rojas, E., and Rudy, B., J. Physiol. *262* (1976) 501.
20 Eaton, D. C., Brodwick, M. S., Oxford, G. S., and Rudy, B., Nature *271* (1978) 473.

0014-4754/89/040346-04$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1989

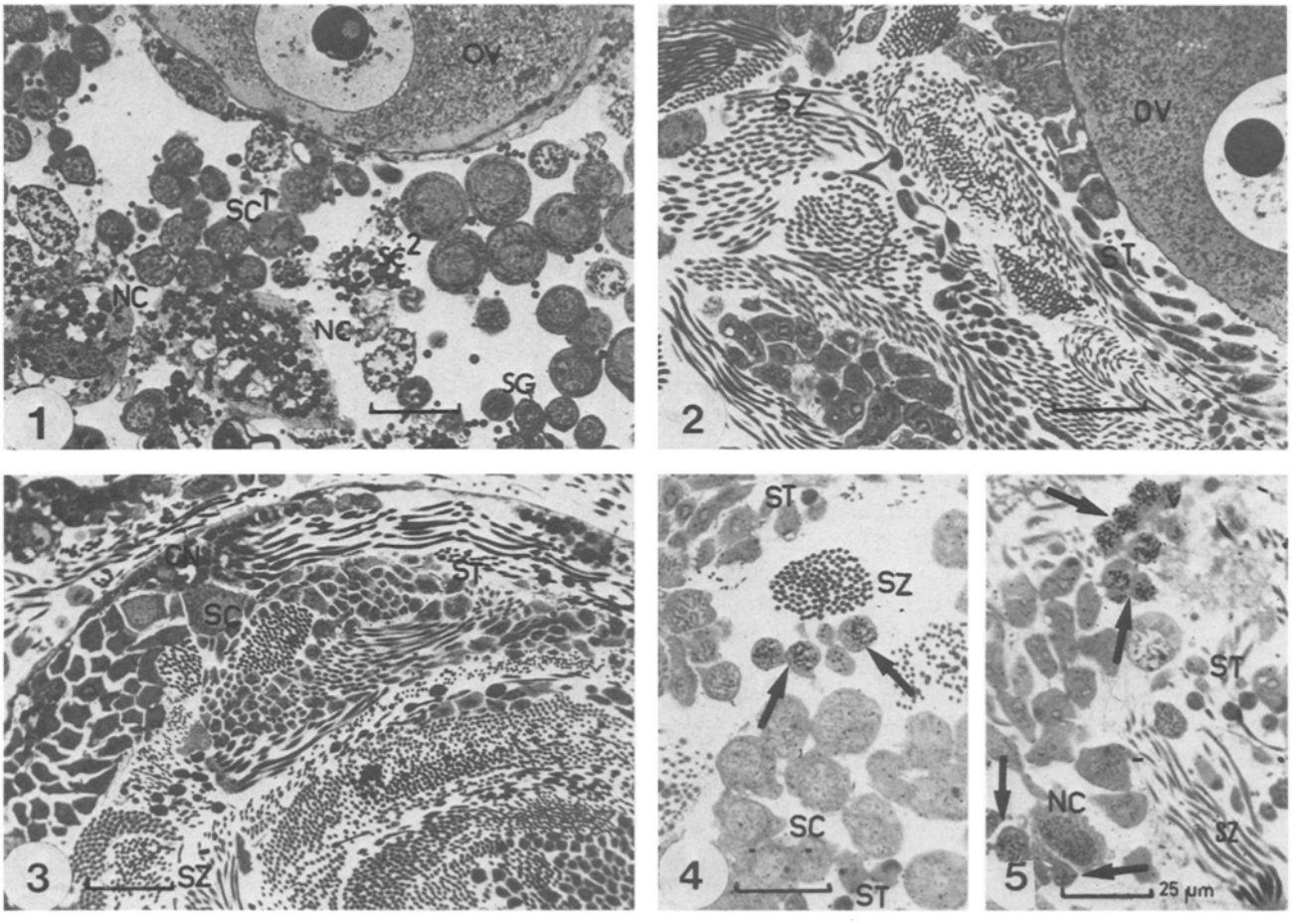
Inhibition of temperature-induced spermatogenic proliferation by a brain factor in hibernating *Helix aspersa* (Mollusca)

P. Gomot and L. Gomot

Laboratoire de Zoologie et Embryologie, URA CNRS 687, Faculté des Sciences, place Maréchal Leclerc, F-25030 Besançon Cedex (France)
Received 2 December 1988; accepted 23 December 1988

Summary. Ablation of the brain from hibernating *Helix aspersa* maintained at 25 °C causes a significant increase in the proliferation of male cells in the gonad, whereas the ablation of the optic tentacles has no effect. The brain, therefore, produces a factor which specifically inhibits the multiplication of spermatogonia and spermatocytes.
Key words. Spermatogenesis; temperature; brain; hibernation; *Helix aspersa.*

Hibernation is a mode of biological adaptation of animals exposed to unfavorable environmental conditions. Normally the pulmonate snail *Helix aspersa* hibernates for five months during fall-winter. When snails in their first few months of hibernation are transferred to optimal conditions which would normally permit them to reproduce[1], they fail to do so[2]. It seems that a refractory period prior to reproduction exists in hibernating snails. Our interest centers on the role of the cephalic neuroendocrine organs of snails (brain and optic tentacles) in the



Figures 1–5. Cytological appearance of the ovotestis of control and experimental snails. At 5 °C there is no difference between the controls and experimental animals (fig. 1). One can see the oocytes (OV), the spermatogonia (SG), the spermatocytes 1 (SC 1) or II (SC 2) and the large nurse cells (NC). At 25 °C spermiogenesis is complete in the controls (fig. 2) and in the brain-ablated snails (fig. 3). The spermatogonia and the labelled spermatocytes (→) are clearly more numerous in brain-ablated snails (fig. 5) than in controls (fig. 4). ST: spermatids; SZ: spermatozoa. Bar equals 25 µm.

control of male gametogenesis. Previous findings in *Cepaea nemoralis*[3] and *Helix aspersa*[4] have shown that a low temperature, for example 6 °C, inhibits whereas 23 °C favors spermatogenesis. In this paper we have studied the effect of the ablation of the brain and the optic tentacles on the proliferation of spermatogonia and spermatocytes and differentiation of spermatids in inactive snails maintained at 5 °C and 25 °C.

Materials and methods. The experiments were carried out in December on adult *Helix aspersa* Müller collected from the Vaucluse at the start of their natural hibernation (November). They were maintained in the lab for 6 days at 5 °C.

Under anesthesia with succinylcholine the supra-esophageal cerebral ganglia (brain) and/or the optic tentacles were removed surgically. After operation, retracted inactive snails (6 in each group) were placed either at 5 °C or 25 °C for four weeks. 5 h before sacrifice each snail received 5 µCi/g of methyl ^{3}H thymidine (sp. act. 1 Ci/mmole) by injection into the hemocoel. Pieces of the gonad and hepatopancreas were fixed for semi-thin plastic sections for cytology and autoradiography[4]. Other pieces of the gonad and hepatopancreas were weighed and hydrolyzed with 0.3 M NaOH for 1 h at 60 °C. An aliquot was used to estimate the DNA content of the tissues by fluorometry using the DAPI-DNA complex method[5]. Another aliquot was chased with unlabelled thymidine for 1 h, 1 mg/ml of DNA was added as carrier, and DNA was then precipitated with 20% perchloric acid for 20 min at 4 °C. The precipitate was collected on a filter and washed[6]. The radioactivity was measured by scintillation counting and expressed either per mg wet weight or per pg DNA of the organs studied.

Results and discussion. A cytological examination of control snail gonads (fig. 2) or gonads from brain ablated snails (fig. 3), placed at 25 °C for four weeks, shows that cell divisions of spermatogonia and spermatocytes and differentiation of spermatids into spermatozoa proceed normally, whereas the gonads of animals kept at 5 °C contain only spermatogonia and spermatocytes, suggesting that in the latter situation cell divisions are arrested (fig. 1). Autoradiography shows that at 25 °C only the

Comparison of ^{3}H thymidine incorporation in the ovotestis and hepatopancreas of brain-ablated and control snails. The incorporation in the ovotestis is 2–2.5 times higher in brain-ablated snails over controls, whereas the incorporation in the hepatopancreas remains unaltered in both experimental and control groups. The values marked with an asterisk (*) are significantly different from control values ($p \leq 0.05$ non parametric U test of Mann and Whitney).

^{3}H incorporation (cell replication)	Organ	Experiment Controls	Brain ablation	Optic tentacle ablation	Brain and optic tentacle ablation
dpm/mg wet weight	Ovotestis	158 ± 14	398 ± 52*	157 ± 38	414 ± 112*
means ± SD	Hepatopancreas	68 ± 14	58 ± 10	48 ± 5	59 ± 18
dpm/pg DNA	Ovotestis	52 ± 6	100 ± 6*	38 ± 6	109 ± 14*
means ± SD	Hepatopancreas	36 ± 6	21 ± 3	13 ± 3	23 ± 13

male cells such as spermatocytes incorporated ^{3}H thymidine (figs 4, 5). In brain-ablated snails, these cells incorporating ^{3}H thymidine were more numerous in the acini (40–60 positive cells/mm^2 section) (fig. 5) than in controls (15–17 positive cells/mm^2 section) (fig. 4). The incorporation of ^{3}H thymidine in the ovotestis as well as in the hepatopancreas in both control and brain-ablated snails maintained at 5 °C was appreciably less and was of the order of 10–17 dpm/pg DNA. On the other hand, in snails of the experimental goup (brain-ablated) maintained at 25 °C the incorporation of ^{3}H thymidine in the ovotestis was appreciably higher than in controls (table 1). The table also shows that the response to higher temperature after ablation of the brain is specific to the male lineage and that the cellular division of a control organ (the hepatopancreas) is not affected. The ablation of the optic tentacles, which exerts an action on the male lineage of the ovotestis *in vitro*[7], is not accompanied by any change in spermatogenic multiplication (table). The simultaneous ablation of the brain and the optic tentacles has similar effects to those caused by the removal of the brain alone (table).

These data lead to the suggestion that at the beginning of hibernation the stimulation of spermatogenic proliferation by high temperature (25 °C) is inhibited by the brain. This inhibition represents one of the probable causes of the delayed reproduction seen during the first month of hibernation. We have also, for the first time, identified the site of action of temperature in the control of male germ cell multiplication, and found that it is located in the brain. The differentiation of spermatids into spermatozoa is independent of the presence of the brain, so the factor responsible for this may be situated elsewhere. The action of temperature on the brain of snails can be compared to that of the photoperiodicity in the brain of insects from temperate regions in their adaptive response to winter conditions resulting in the diapause[8]. In the future we hope to perform ablation experiments involving different parts of cerebral ganglia and also surgically to remove the endocrine dorsal bodies which are closely associated with cerebral ganglia, in order to determine the precise location of the sensitive zone(s) in the brain of *Helix aspersa* which apparently produces a factor inhibiting male cell multiplication.

The authors wish to thank Claude Colard for technical help and Brigitte Jolibois and Félix Sanchez for the organization and the illustration of the manuscript. We appreciate the help of A.S.M. Saleuddin for the critical reading of the manuscript.

1 Gomot, L., and Deray, A., La Recherche *18* (1987) 302.
2 Bonnefoy-Claudet, R., and Deray, A., C.r. Soc. Biol. *178* (1984) 442.
3 Bouillon, J., Nature *177* (1956) 142.
4 Gomot, P., Griffond, B., and Gomot, L., C.r. Acad. Sci. Paris, ser. III *302* (1986) 27.
5 Brunk, C. F., and Jones, K. C., Analyt. Biochem. *92* (1979) 497.
6 Lasalle, B., Biol. cell. *47* (1983) 187.
7 Gomot, L., Archs Anat. Hist. Embryol. norm. exp. *56* (1973) 131.
8 Lavialle, M., and Dumortier, B., C.r. Acad. Sci. Paris, ser. III *307* (1988) 117.

0014-4754/89/040349-03$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1989