Neurosecretory action potentials recorded extracellularly from a neurohemal region associated with the Y-organ in the terrestrial isopod, *Oniscus asellus*

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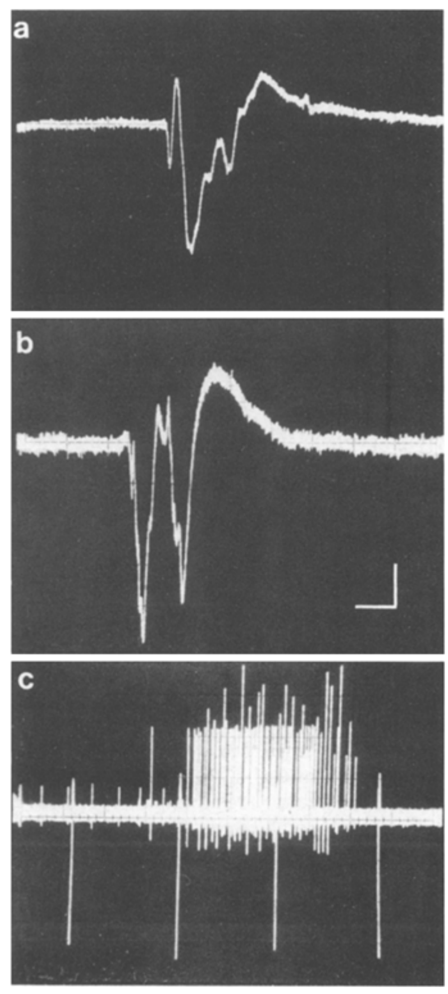
Summary. A long duration (over 30 ms), large amplitude (up to 1000 μV) action potential was recorded by an extracellular electrode placed near the Y-organ, or molting gland, of the terrestrial isopod, *Oniscus asellus*. This action potential discharged in a slow tonic fashion (0.20–40.0 spikes per min), and eminated from the lateral nerve plexus (LNP), a neurohemal region believed to control, in part, the activity of the Y-organ. By correlating the rate of electrical activity of the LNP with the molt cycle, it will be possible to assess the role of these neurosecretory cells in regulating the production of ecdysone by the Y-organ.

Key words. Lateral nerve plexus; neurosecretion; isopod; extracellular potentials.

It is known that the molting gland or Y-organ of isopod crustaceans is associated with a large nerve plexus (LNP) believed to contain terminals and cell bodies of neurosecretory cells (NSCs) controlling, in part, the rate of ecdysone produced by the Y-organ[1–9]. However, despite these numerous investigations of the structure of the LNP in isopod crustaceans, electrophysiological studies have yet to be undertaken to determine if electrical activity could be recorded from the LNP associated with the Y-organ, and if so, could this activity be correlated with the molt cycle. In an electrophysiological study conducted on the molting gland or prothoracic gland (PTG) in the insect, *Periplaneta americana*, a correlation was found between the electrical activity associated with the PTG and the ecdysteroid titer in the hemolymph[10]. This correlation suggested a stimulatory nervous input for ecdysone production. It seemed profitable, therefore, to determine if electrical activity could be recorded from the Y-organ of the terrestrial isopod, *Oniscus asellus*. These isopods are ideal candidates for correlating electrical activity with the stages of the molt cycle: their molt cycles are well defined, premolt is initiated with the appropriate environmental cues[11], and their ecdysteroid titers have been determined throughout the molt cycle (Steel, unpublished observations). Furthermore, electrophysiology of their sinus glands, which are important neurosecretory structures in the head, have also been documented, and correlations were found between NSC types and identified action potential types[12], and between the overall electrical activity and the stages of the molt cycle[13].

Materials and methods. For electrophysiological recordings, whole animals were secured in modelling clay with the anterior side of the head facing upwards and brains exposed as previously described[12]. Y-organs were located on each side of the head directly ventral to the compound eyes, juxtaposed on the ventral exoskeleton near the musculature controlling the mandibles. An extracellular suction electrode with a tip diameter of 10–50 μm was filled with physiological saline and positioned under the compound eye near the Y-organ (see Chiang and Steel[12] for details). Electrical activity was recorded with respect to an indifferent electrode placed in the pool of hemolymph over the exposed region of the head. Electrical activity was amplified with a WPI DAM-5A differential preamplifier, viewed on an oscilloscope screen, and recorded in digital format on video tape for further analysis[14].

Results and discussion. The extracellular electrode, placed within a few μm of the Y-organ, recorded a tonically discharging action potential. This action potential displayed a complex wave form with total deflections ranging from 400 to 1000 μV, and durations of over 30 ms (fig. a). The appearance of the complex wave form was relatively consistent within a preparation. While its amplitude decreased as the electrode was moved away from the Y-organ, it still maintained the same general shape. This shape varied to a limited extent between preparations. In all cases, the most prominent portion of the action potential was a large rapid downward deflection usually followed by a slightly slower upward deflection that overshot the baseline before slowly returning



Sample oscilloscope traces of extracellularly recorded action potentials eminating from the lateral nerve plexus, a neurohemal region associated with the Y-organ in terrestrial isopods. *a* Electrical activity from a lateral nerve plexus discharging a single compound action potential. *b* Electrical activity from a lateral nerve plexus discharging two closely coupled compound action potentials. *c* Extracellular action potentials eminating from the sinus gland (upward deflections) and the lateral nerve plexus (four large downward deflections) as recorded by an electrode placed over the sinus gland. The tonic discharge of the lateral nerve plexus action potential contrasts sharply with the phasic bursts of action potentials from the sinus gland. Sinus gland potentials were recorded only if the sinus gland were drawn into the suction electrode[12]. Scale mark: vertical, 100 μV; horizontal, 10 ms in *a*, 20 ms in *b* and 2 s in *c*.

to zero. A number of smaller oscillations from the baseline immediately preceded and/or followed the large downward deflection. These oscillations may represent discharges of other electrically active units which are closely coupled to the large downward deflection. In some of the preparations examined, the electrical activity consisted of two tonically discharging action potentials. Each displayed the general shape described above. Their discharges were closely coupled and occurred within 5–60 ms of one another (fig. b).

The LNP is located in close proximity to the anterior region of the Y-organ, and the largest amplitudes were recorded at this location, thus verifying that the compound action potential was generated by the LNP. Moreover, various characteristics of this compound action potential can be correlated with known features of the LNP. For instance, the LNP is formed by the junction of several nerves, and serves as a neurohemal region containing terminals of NSCs whose cell bodies are located in the brain[9]. As seen in the present electrophysiological study, the action potential displayed a long duration indicative of NSC axons and terminals[15], and possessed a multi-component wave form reflecting the presence of a number of electrically active units. Transection of pathways linking the Y-organ to the brain also eliminated electrical activity indicating that the initiation of spontaneous activity occurred in regions outside the LNP. This result would be expected if the perikarya were located in the brain. The tonic discharge of the LNP differed from the discharge pattern of neurosecretory action potentials recorded from the sinus gland of the same animal (fig. c). Whereas electrical activity recorded from the sinus gland occurred in bursts with interburst frequencies as high as 90 Hz[12], the electrical activity associated with the Y-organ discharged in a slow tonic fashion ranging from 1 every 5 min to, at most, 40 per min. The tonic discharge suggests a sustained release of neurohormones. Such a release would be advantageous for a NSC whose role is to maintain a long-term inhibitory (or excitatory) control over the activity rate of an endocrine gland.

The above results demonstrate that electrical activity characteristic of NSCs can be recorded by an extracellular electrode placed near the Y-organ of terrestrial isopods. This activity eminates from the neurosecretory terminals in the LNP making it possible to compare the electrical activity of the LNP with that of the sinus gland which is also believed to release substances controlling Y-organ activity[16]. A description of the electrical activity of the LNP throughout the molt cycle will therefore provide additional insight into the neuroendocrine processes controlling molting in crustaceans.

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Effect of oridonin, a *Rabdosia* diterpenoid, on radiosensitization with misonidazole

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Summary. The radiosensitization brought about by oridonin, one of the *Rabdosia* diterpenoids, alone or in combination with misonidazole, was investigated in Chinese hamster V79 cells. The enhancement ratio of 1.92 was obtained when 0.01 mM oridonin and 1 mM misonidazole were administered to hypoxic cells under radiation. The enhancement ratios of oridonin and misonidazole for hypoxic cells were 1.16 and 1.59 respectively. Hence, a supra-additive effect was obtained by the combined treatment with these two drugs. Under aerobic conditions, no effect of 0.01 mM oridonin on the radiosensitization caused by misonidazole was observed.

Key words. Oridonin; *Rabdosia* diterpenoid; thiol-reactive agent; misonidazole; radiosensitizer.

Misonidazole (fig. 1, **1**) is known to be an effective radiosensitizer for hypoxic cells; clinical trials of the drug are being conducted in a number of countries throughout the world. In spite of a slight benefit in some tumor sites, the dose of misonidazole is generally too low to attain efficient sensitization of hypoxic tumor cells, because of its neurotoxicity[1,2]. In order to avoid the neurotoxic properties of misonidazole, there has been a great effort to develop new radiosensitizing drugs which are less lipophilic or more rapidly excreted[3,4]. Another appraoch has started; that is, to search for agents that will enhance the efficiency of existing radiosensitizers, hopefully without increasing their toxic side effects. This involves decreasing the endogenous radioprotective capacity by depleting glutathione (GSH) and other intracellular free thiols (e.g. cysteine), because such thiol compounds compete with radiosensitizers for repair or fixation of radiation-induced damage. Recently, several in vitro and in vivo studies have been reported in which diethyl maleate (DEM, a thiol alkylating agent) or buthionine sulphoximine (BSO, an inhibitor of GSH synthesis) has been used to deplete intracellular GSH; the radiosensitizing effectiveness of misonidazole has been improved by combining it with those drugs[5,6].

In 1970, Fujita's group[7] isolated a kaurene-type diterpene, oridonin (fig. 1, **2**), from *Rabdosia trichocarpa* Kudo (Labia-