Effect of Colchicine on Long Lasting Synaptic Modifications Induced by Hyperactivity

The exaggerated use of antigravity reflexes, induced by loading a cockroach, results in a modification of 'synaptic permeability', i.e., an increase in the probability of transmission along a particular synaptic pathway[1]. Such modification has been shown to be a long lasting process that persists, after unloading the insect, for periods of time directly releated with the duration of the loading[2,3]. Recently, it has been suggested that synaptic changes, and related chemical events, may be dependent upon molecules synthesized in the perikaryon and axonally transported to the synaptic endings[4]. The present work was designed to study the effect of colchicine, an alkaloid known to block axoplasmic transport[5,6], on the aforementioned increase in synaptic permeability.

Material and method. Adult female cockroaches (*Blatta orientalis*) were anesthetized with CO_2 and the first thoracic ganglion exposed by a small incision of the overlying chitin. From a micrometer syringe, 0.2 µl of physiological saline either alone or containing 10 m*M* colchicine was injected under the epineurium of this ganglion through a 1-µm tipped pipette[7]. More than 95% of the animals survived this operation over the usual testing periods (1–72 h) and their response to tactile stimulation could not be distinguished from that of untreated animals. At different time intervals after injection, pieces of lead were attached to the insects' dorsal surface so as to double their weight[1]. In all the experiments, such a load was maintained in place for 3 h and the testing of synaptic transmission was performed 3 h after unloading.

Synaptic transmission was tested in vitro by dissecting out the length of the nerve cord from the suboesophageal to the 4th abdominal ganglion, including the fifth nerves[8] of the metathoracic ganglion. Single supramaximal stimulating shocks (0.5 msec duration; 1/sec repetition rate) were applied to the pre-synaptic element at the connectives between the oesophageal and the first thoracic ganglion. Propagated all-or-none responses were recorded from the post-synaptic element by means of bipolar electrodes, made out of fine platinum wire, placed in contact with the fifth nerve[9]. In all the experiments the evaluation of synaptic permeability was made, in keeping with other reports[2,3,9], by calculating the percentage of preparations giving a propagated post-synaptic response.

Results and discussion. In 100 control preparations, in which an injection of physiological saline alone was performed just before loading, the percentage of preparations giving a post-synaptic response was about 80–85%, between 6 and 72 h after the injection (Figure). As has been reported, uninjected cockroaches also give a response in about 80–85% of the cases, whereas in normal unloaded roaches this percentage is approximately 10%[1-3]. On the other hand, in 132 experiments, in which a sham operation was performed and the insects were not loaded, the percentage was 93% after 1 h. However, this value gradually decreased with time, and 24 h later it was similar to that obtained from normal cockroaches (Figure).

In contrast to the aforementioned results, in 115 preparations, when colchicine was injected prior to loading, the number of preparations showing a response decreased, reaching the lowest value between 6 and 24 h after the injection. This decrement clearly showed inhibition of the synaptic facilitation observed in the control series. The maximum inhibition was 90–100% at 12 h and diminished after 24 h; at 72 h the effect of the drug was no longer present, i.e., this effect was reversible (Figure). In turn nerve conduction was not affected, as shown by the fact that using the site of stimulation mentioned (see method), action potentials were recorded from the connectives between the first and second abdominal ganglion. This finding is in agreement with those showing that colchicine does not affect nerve conduction in other preparations[10,11].

These experiments on the cockroach show that colchicine reversibly blocks the increase in synaptic permeability induced by loading the insects. Concerning the way in which this result is brought about, the drug could be acting directly on: a) the synapse implicated in such process; or b) indirectly by blocking axoplasmic transport. The former would be compatible with a report showing that colchicine, injected intraocularly in the pigeon, induces post-synaptic depression in the optic tectum[11]. However, in the present study the drug was applied locally and its action most probably did not extend from the site of application to the particular synapse involved. This is reinforced by the fact that the effect of colchicine injection into an abdominal ganglion of either the cockroach[12] or the crayfish[5] is confined to approximately a 3 mm length of nerve cord around the treated ganglion. In the roach nerve cord, the distance between the site of injection and the metathoracic ganglion is of the order of 6–8 mm.

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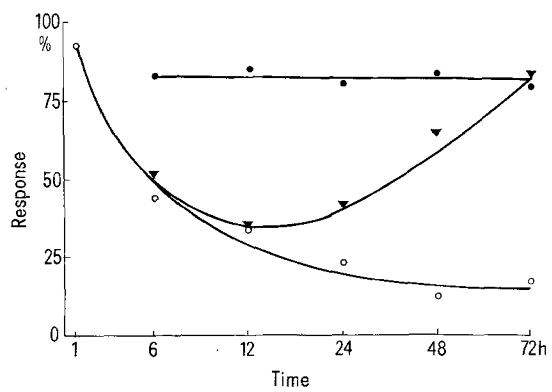

Ordinate: percentage of preparations giving an all-or-none propagated post-synaptic response. Abscissa: time after treatment (hours). Experimental conditions: ●, injection of saline alone (each point, 20 preparations); ▼, injection of colchicine (each point, 23 preparations); ○, sham operation and insects not loaded (each point, 22 preparations).

In view of the demonstrations that colchicine interferes with axoplasmic transport in a variety of preparations[5,6,13], including the cockroach nervous system[12], we favour the second possibility mentioned above, namely, that the present findings are the result of axoplasmic transport blockage by colchicine. In this regard, at least 3 rates of protein transport (1, 24 and 72 mm/day) have been shown in the cockroach[14]. Whether any of these proteins are involved in the synaptic changes described is not yet known; further studies are in progress in order to elucidate the problem.

Resumen. La facilitación de transmisión sináptica en un ganglio de cucaracha se puede inducir mediante hiperuso de reflejos anti-gravitatorios. La inyección intraganglionar de colchicina inhibe reversiblemente la modificación sináptica sin alterar la conducción nerviosa. Este resultado puede explicarse considerando que la colchicina bloquea el proceso de progresión axoplasmática.

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Responses to Osmotic Concentration Changes in the Lobster Antenna

It is well known that stenohaline Crustacea reside in an environment with limited variability of concentration and that they avoid unsuitable osmotic conditions. Although the presence of osmoreceptor is presumed, a specific organ sensitive to changes in osmotic pressure has not been identified. Motor responses have been studied in a marine crab when the antennule is stimulated with various concentrations of sea water[1]. Chemoreceptor responses in Decapods have been reported by many authors[2–4], but water responses, such as are described in vertebrate chemoreceptors[5,6], have not been observed. This paper communicates afferent responses to osmotic concentration changes of various solutions in the lobster antenna.

Material and methods. The antenna of the lobster *Panulirus japonicus* consists of stout basal segments and a long flexible flagellum. Recordings of afferent impulses from antennal nerves were performed on the isolated flagellum of about 5 cm in length. Two recording electrodes (silver wire covered with a glass capillary except for the wire tip) were introduced into the lumen from both the cut ends respectively. Impulses were picked up on the tip of electrode and displayed on a cathode-ray oscilloscope through a CR amplifier (time constant, 0.01 sec). Among chemicals tested were NaCl, choline-Cl, sucrose and glycerol. These test solutions were made up in the pure water of various concentrations. A small amount of the test solution (0.2 ∼ 0.4 ml) was applied to the cuticular surface of the antenna by means of a small glass pipette. After stimulation, the material was washed with sea water. All the experiments were carried out at room temperatures (20 to 22 °C).

Results and discussion. The application of pure water gave rise to a high-frequency volley of impulses for several seconds. The water sensitive organ was found to be a small seta with many hairs on its tip found in great numbers distributed over the exoskeleton of the flagellum.

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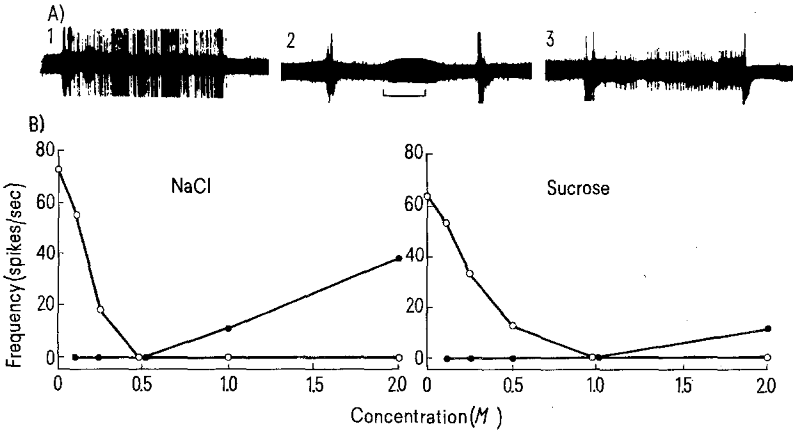

Responses to osmotic concentration changes. A) electrical recordings of responses to stimuli of NaCl solutions. 1. 1/8 *M*; 2. 1/2 *M*; 3. 3.1 *M*. 1/2 *M* NaCl is osmotically equivalent to sea water. Large and small units were evoked at hypotonic and hypertonic concentrations, respectively. Large deflections of traces before and after the responses indicate stimulus and wash artifacts. Calibration: horizontal bar, 1 sec. B) responses partly shown in A were plotted with frequency against concentration of NaCl or sucrose. Open circle, large unit; solid circle, small unit.