suggests that the morphine-induced hyperlactacidemia results largely from anerobic muscle glycogenolysis which is mediated by β-adrenergic receptors. On the other hand, it seems that α-receptors are not involved since phentolamine has no effect of its own nor does it modify the action of morphine.

Hyperlactacidemia is a constant metabolic symptom in morphinized animals, even more interesting as it persists after cessation of morphine treatment. In our experimental series blood lactate concentration remains significantly higher than control level up to 10 days after stopping an 8-day morphine treatment. Given the permeability of the blood brain barrier to lactate[10], an accumulation of lactate in the blood can result in a higher concentration in the brain as has been shown in morphinized rats[3]. Abnormally high brain lactate concentration might be related to the state of anxiety[11] found in drug addicts under withdrawal. We have shown that hyperlactacidemia persists after withdrawal from morphine and that it is completely suppressed by propranolol. This is very interesting because of recent reports demonstrating the effectiveness of propranolol in treating anxiety in man[12,13] and heroin addicts[14].

Table II. Effect of propranolol (5 mg/kg) on blood lactate in fed (N) and 15 h fasted (F) rabbits

Lactate (mg p. 100 ml plasma)				
Time	0	1	3	6 hours
1st injection				
F (8)	15.6 ± 1.9[1]	14.6 ± 1.1	17.7 ± 3.2	15.0 ± 2.4
N (8)	34.1 ± 5.3[2] $p < 0.01$ [a]	29.3 ± 3.8	29.7 ± 4.4	28.5 ± 3.4
10th injection				
F (8)	14.4 ± 0.9	16.9 ± 2.0	14.3 ± 1.1	16.4 ± 1.9
N (8)	16.4 ± 2.3 $p < 0.01$ [b]	17.2 ± 0.8	17.4 ± 2.4	20.3 ± 2.1

Values are means $\pm$ SE; p is given by Student's t-test. [a] vs (1); [b] vs (2). The number in parentheses represent the number of animals.

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Frescon: Neurophysiological Action of a Molluscicide

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Summary. The molluscicide N-trityl morpholine ('Frescon') has an unusual effect on the central nervous system of a freshwater snail. Nerve impulses become grouped into spontaneous 'bursts', with many cells firing synchronously. This may result from interference with inhibitory processes.

N-tritylmorpholine (Frescon, Shell Chemicals) is toxic to many freshwater snails[3]. It is highly specific, being harmless to plants, insects and most vertebrates, though it is moderately toxic to some species of fish[4]; even the terrestrial Gastropod *Helix aspersa* is apparently immune[4]. It is therefore important in controlling the snail hosts of a number of parasites, including species of *Schistosoma* which give rise in man to the widespread tropical disease, bilharzia[5].

Frescon is lethal to freshwater pulmonate Gastropods at very low concentrations (e.g., *Biomphalaria glabrata*: LD_{50} (24 h) $= 2.5 \times 10^{-8}$ g/ml[6]). *Lymnaea stagnalis* is killed by 10^{-6} g/ml Frescon in 3 h at 20 °C. Its specificity and rapid effect have caused speculation as to its mode of action.

Investigations so far have not suggested any interference in such metabolic processes as oxidative phosphorylation[4]. Preliminary experiments have however suggested that Frescon may cause abnormalities in the electrophysiology of the *Lymnaea stagnalis* nervous system[4]. To investigate this further, individual nerve cells from the visceral or right parietal ganglia of the isolated central nervous system of this species were impaled by 1 M potassium acetate microelectrodes (ca. 25 $M\Omega$) using standard electrophysiological recording techniques. Separate recording and stimulating electrodes were used to allow control of membrane potentials. Normal Ringer solution[7] consisted of 50 mM NaCl, 1.6 mM KCl, 4 mM $CaCl_2$, 2 mM $MgCl_2$ and 5 mM *Tris*-Cl (pH 8.0) in distilled water. In more recent experiments, 50 mM sucrose was also added to provide a better osmotic balance. Low Ca/high Mg Ringer contained 2 mM $CaCl_2$ and 20 mM $MgCl_2$, with 23 mM sucrose to maintain overall osmolarity. As Frescon is very hydrophobic, it was used in a di-

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methylsulphoxide and detergent formulation (FX 2574, Shell Chemicals) containing 10^{-2} g/ml Frescon. Immediately prior to use this was diluted with Ringer solution producing a fine suspension. Nominal Frescon concentrations of 10^{-6} or 10^{-5} g/ml were used. Control experiments with the blank formulation showed no effect.

Figure 1 shows the electrical potentials of a *Lymnaea* nerve cell before, and 63 min after exposure to 10^{-6} g/ml Frescon. Resting and action potentials are unaffected, but the membrane potential is suddenly depolarized by a huge summating burst of synaptic potentials. 75% of all neurones exposed to Frescon show these typical 'Frescon bursts'. The first incidence usually occurs after

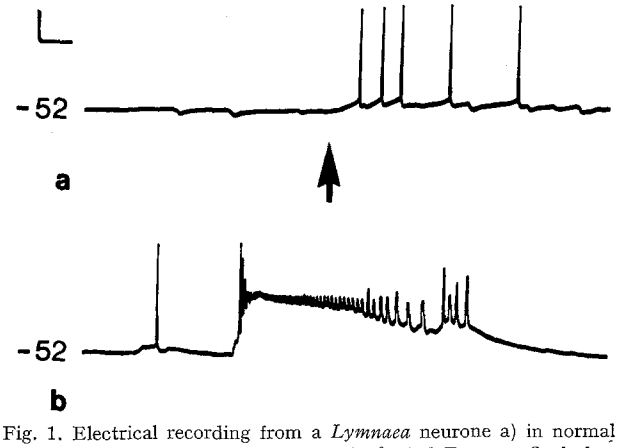

Fig. 1. Electrical recording from a *Lymnaea* neurone a) in normal Ringer, b) after 63 min exposure to 10^{-6} g/ml Frescon. Scale bar 20 mV (vertical), 250 msec (horizontal). Numbers at left give the resting membrane potential in mV. Action potentials in a) were elicited by a 3 mV depolarization (arrowed).

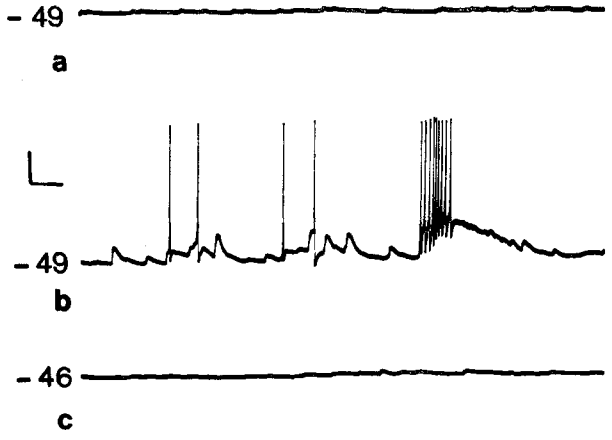

Fig. 2. a) Normal Ringer, b) after 53 min exposure to 10^{-5} g/ml Frescon, c) after 5 min in low Ca/high Mg Ringer + Frescon. Scale bar 20 mV, 500 msec. Resting potentials in mV.

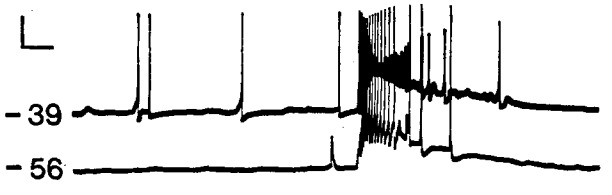

Fig. 3. Simultaneous recording from 2 neurones, after 33 min exposure to 10^{-5} g/ml Frescon. Scale bar 20 mV, 500 msec. Resting potentials in mV; vertical positions of the two traces adjusted to give a clear record.

10–20 min exposure to Frescon; thereafter 'bursts' appear spontaneously and at random. The effect appears to be irreversible even after prolonged washing with Ringer + dimethylsulphoxide. There is no gradual or consistent increase in size or frequency of synaptic potentials prior to a 'Frescon burst'. Over a period of 1–2 h, however, there is often a general increase in synaptic activity (cf. Figure 2a and b) with the 'Frescon bursts' becoming more frequent.

'Frescon bursts' cannot be elicited by intracellular stimulation, and do not appear to be due to a direct effect on somatic resting or action potentials, which remain unaltered. A synaptic origin of the 'bursts' is indicated by the effects of a low Ca/high Mg Ringer (Figure 2), which is generally found to reduce normal quantal release of transmitter substances[8]. The synaptic potentials are greatly reduced in amplitude, with a corresponding decrease in frequency of the 'bursts'. Simultaneous recordings from separate neurones indicate that all cells in a preparation which exhibit 'Frescon bursts' do so synchronously (within 10–200 msec of each other), never independently (Figure 3).

These results therefore raise the possibility that Frescon, perhaps by dissolving in the lipid phase of the cell membranes, could be interfering with either the presynaptic vesicular release mechanism or the efficacy of the transmitter in changing postsynaptic membrane conductances. In the former case Frescon could be producing a massive release of transmitter. However it is difficult to see how this could produce the observed discontinuous activity represented by the 'Frescon bursts': for instance, Black Widow Spider venom, which has been shown to interfere with presynaptic vesicle release[9], simply produces a continuous increase in synaptic potential frequency followed by an exponential decline back to control levels.

A specific postsynaptic potentiation, on the other hand, was also considered unlikely, as the Frescon effect did not seem to be limited to any one transmitter substance (and by inference, receptor complex): neurones depolarized by topical application of acetylcholine, 5-hydroxytryptamine, or noradrenaline were all capable of exhibiting 'Frescon bursts', as were cells which were hyperpolarized by acetylcholine. Occurrence of 'Frescon bursts' in a large proportion of cells in the ganglion also argues against specific transmitter involvement.

A third possibility is that Frescon modifies the action of synaptic networks in such a way as to cause intermittent massive discharges affecting the entire nervous system. This 'synaptic network facilitation' could perhaps be achieved by a small change in the balance of excitatory and inhibitory inputs to each cell. So far the evidence obtained is compatible with this hypothesis (for instance, note the apparent absence of inhibitory postsynaptic potentials after Frescon exposure (Figure 1)). Thus the reduction of the Frescon effect in low Ca/high Mg Ringer is explained by the general diminution of all synaptic potentials throughout the neuropile due to reduced vesicular release of transmitter. Also the synchrony of the Frescon effect suggests a total nervous system involvement, rather than changes to specific isolated synaptic terminals.

The nervous system therefore appears to be a possible site for Frescon action in freshwater snails. Confirmatory experiments are in progress.

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