Action of heptaminol hydrochloride on contractile properties in frog isolated twitch muscle fibre

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- 1 Heptaminol stopped or delayed the progressive decline in tension which characterizes the phenomenon of fatigue in frog isolated twitch muscle fibre.
- 2 Heptaminol had no action on the sodium, potassium and calcium voltage-dependent ionic conductances.
- 3 The hypothesis of an action via an internal alkalinization was tested by comparison with the action of NH_4Cl . Both substances increased the tension.
- 4 The action of heptaminol was suppressed in sodium-free (TRIS) solution or in the presence of amiloride while the action of NH_4Cl was always observed.
- 5 These results could be explained by a stimulation of the Na/H antiport by heptaminol.

Keywords: Heptaminol; fatigue; excitation-contraction coupling; frog twitch muscle fibre.

Introduction

Heptaminol (6-amino-2-methyl-2-heptanol) hydrochloride (Heptamyl, Delalande) is primarily known as a potent cardio-tonic agent (Loubatières, 1949). In animals as well as in man (Rigal *et al.*, 1963), early work showed that heptaminol may prevent fatigue in nervous and muscle system activities. Intra-peritoneal injection of the drug in exhausted rat restored normal values of neuronal and muscular chronaxies (Chauchard & Mazoue, 1958). In dog heart, hypotensive and depressant effects of (+)-tubocurarine were minimized in the presence of the drug (Loubatières *et al.*, 1961). It has been reported (Coraboeuf & Boistel, 1953) that heptaminol increased action potential plateau, contraction amplitude and spontaneous activity frequency in nodal tissue; toxic effects of high CO_2 concentrations were reduced in its presence and a normal electrical activity was restored in exhausted cardiac tissues. Müller *et al.* (1964) observed similar potentiating actions on contraction in mammalian ventricular trabeculae; these authors mentioned that action potentials and Na/K pump activity were not modified.

The positive inotropic action of heptaminol is therefore well established; nevertheless the underlying cellular mechanisms remain unknown. Heptaminol could act at different steps involved in the process of excitation-contraction coupling or in the control of the function of the myofilaments. It has been shown on squid axon (Vassort *et al.*, 1986) that alcohol and amine groups can induce an increase in internal calcium and a decrease in internal protons. On the other hand it is well known that the calcium sensitivity of myofilaments and, in some way, the sarcoplasmic reticulum calcium release are increased during intracellular alkalinization (Fabiato &

Fabiato, 1978). Heptaminol which has both an alcohol and an amine group could produce its positive inotropic effect by an internal alkalinization. Such an explanation has recently been envisaged by Berthiau *et al.* (1989) who suggested that the inotropic effect induced by heptaminol on ischaemic rat myocardium could be due to stimulation of the Na/H antiport.

In the present paper, experiments have been performed to investigate the effect of heptaminol on the electrical and mechanical responses of frog isolated twitch muscle fibre in conditions of repeated stimulation inducing fatigue. The main result of this study indicates that the progressive decline in tension which characterizes the phenomenon of fatigue during repeated stimulation was stopped or delayed in the presence of heptaminol.

An action of the drug on the regulation of the intracellular pH is discussed. Some of the results have been published in abstract form (Allard & Rougier, 1989a).

Methods

The experiments were performed at room temperature on intact isolated single fibres from the semitendinosus of the frog *Rana esculenta*.

Ionic currents, action potentials and isometric tension were recorded in the test gap (200 μm in length) of a double mannitol gap device as previously described in detail by Caille *et al.* (1978) and Potreau & Raymond (1980, 1982). The recording solutions used are shown in Table 1.

Tetrodotoxin (TTX) from Sigma, amiloride from Sigma and 5-(N-ethyl-N-isopropyl) amiloride (EIPA) were added in some experiments. EIPA was a gift from Prof. M. Lazdunski. Heptaminol (Hept-a-myl) and tuamine were supplied by Laboratoires Delalande.

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Table 1 Composition of the recording solutions used (all concentrations mM)

Solution	NaCl	LiCl	CaCl ₂	KCl	MgCl ₂	TRIS	TEA-CH ₃ SO ₃	NH ₄ Cl	4-AP
Control Ringer	110	/	1.8	2.5	2	10	/	/	/
Li Ringer	/	110	1.8	2.5	2	10	/	/	/
NH ₄ Cl Ringer	90	/	1.8	2.5	2	10	/	20	/
0 Na Ringer	/	/	1.8	2.5	2	120	/	/	/
0 Na NH ₄ Cl Ringer	/	/	1.8	2.5	2	100	/	20	/
TEA-CH ₃ SO ₃ Ringer	/	/	1.8	2.5	2	10	122	/	3

TEA = tetraethylammonium; 4-AP = 4-aminopyridine.
In all solutions the pH was adjusted to 7.4.

At the beginning of each experiment the preparations were stimulated at a low rate to permit stabilization. Then different patterns of repeated stimulation were used to induce fatigue.

In current clamp experiments, the fibres were stimulated every minute by a 2 s tetanic train of depolarizing pulses (1 ms, 1.2 rheobase, 50 Hz).

In voltage clamp experiments, several patterns of stimulation were used; they are given in the results section. The holding potential was chosen so that the early sodium current reversed for a depolarization of 120 to 130 mV. In this condition the holding potential of the fibre could be assumed to have a value of -90 mV (see also Caille *et al.*, 1978).

Currents, potentials and tension were simultaneously chart recorded (Gould-Brush 220 or 2400), visualized on an oscilloscope (Textronix 565) and photographed (Camera Nihon-Kohden PC-3A). In some experiments, the records were digitized with a PCM converter (Sony 701) and stored on a video tape for further analysis.

Results

Action of heptaminol on mechanical tension in control Ringer solution

Figure 1a illustrates the contractile behaviour of a preparation under conditions of repeated stimulation in control Ringer solution. In this experiment, after an initial period of stabilization, the preparation was stimulated by voltage clamp depolarizing pulses (100 mV, 200 ms) at a frequency of 12 per min. The amplitude of tension decreased progressively during the course of the experiment. This decline which can be considered as an indication of the 'fatigue' of the preparation differed from fibre to fibre (Figure 1a and b) and varied between 20% to 40% during the first 10 min of the experiment with this pattern of stimulation.

Figure 1 Action of heptaminol on tension during repeated stimulation in control solution. (a) Control experiment showing the progressive decline in tension which characterizes the phenomenon of fatigue. The preparation was stimulated by voltage clamp depolarizations (100 mV, 200 ms) at a frequency of 12 min^{-1} . (b) In this fibre, tension stopped declining during the addition of heptaminol (10^{-4} M). Same protocol of stimulation as in (a). (c) Note that in this fibre which developed a weak contraction, addition of heptaminol (10^{-4} M) increased the tension. The preparation was stimulated by voltage clamp depolarizations (100 mV, 1 s) at a frequency of 3 min^{-1} . (a), (b) and (c) were recorded from different fibres.

Addition of heptaminol (10^{-4} M) increased the tension or stopped its progressive decline (Figure 1b). When heptaminol was removed the tension decreased again. Similar results were obtained in 6 other fibres.

When heptaminol was added after a longer period of repeated stimulation which induced a decrease in tension greater than 50%, a marked increase in tension was always observed (Figure 1c, 4 experiments).

Similar observations were obtained on tetanic tension experiments performed in current clamp conditions (Figure 2). The preparation was stimulated at a tetanic rate as described in the methods section; it generated a train of action potentials (Figure 2a and b, upper row) and developed a tetanic contraction (Figure 2a and b, lower row). This tetanic tension was composed of two phases: an initial peak followed by a plateau. The amplitude and the time course of the plateau differed from fibre to fibre as illustrated in Figure 2a and b. Addition of heptaminol (10^{-4} M) induced a progressive increase in the plateau of the tetanic tension. The potentiating effect became maximal after 4 to 6 min. Action potentials were not appreciably modified, except for a slight decrease in spike amplitude; this was true for the envelope of the action potentials (Figure 2a and b) as well as for single corresponding records (not illustrated).

These results indicate that heptaminol is able to stop or to reverse the appearance of fatigue in an isolated fibre submitted to repeated stimulation.

In order to study the possible mechanism of action of heptaminol, experiments were first performed to test an eventual action on the ionic conductances which are implicated in the process of excitation-contraction coupling (see Caille *et al.*, 1985; Raymond, 1989 for reviews).

Does heptaminol act on ionic currents?

In Figure 3a the preparation was depolarized to $E = -30$ mV by 10 ms duration pulses at a frequency of 1 per second; it developed a large inward sodium current which was not sig-

Figure 2 Action of heptaminol on action potentials and tetanic tension. Note that the time course of control tetanic contraction is different in two different fibres (a and b). When heptaminol (10^{-4} M) was added, the plateau of tetanic tension (lower row, (i), (ii) and (iii)) increased while the train of action potentials (upper row) was not modified. The preparations were stimulated by a 2 s train of depolarizing currents (1 ms, 1.2 rheobase, 50 Hz) at a frequency of 1 min^{-1} .

Figure 3 Action of heptaminol on sodium and potassium ionic current. (a) Action on sodium current. The sodium inward current which was activated for a depolarization to $E = -30$ mV was not modified by heptaminol (10^{-4} M). (b) Action on potassium current. The potassium delayed outward current which was activated for a depolarization to $E = +30$ mV was not modified by heptaminol (10^{-4} M). (a) and (b) were from the same fibre. The preparation was stimulated at a frequency of 1 s $^{-1}$.

nificantly modified after the addition of heptaminol (10^{-4} M). In Figure 3b the same fibre was depolarized to $E = +30$ mV; at this potential close to the sodium current reversal potential, the current which corresponded to the delayed outward potassium current was not modified by heptaminol (10^{-4} M). Similar results were obtained in 3 other fibres. Increasing the concentration of heptaminol (10^{-2} M) induced a decrease of both these membrane currents (not illustrated). This action is certainly due to the general anaesthetic properties of the alcohol group of the molecule.

The following experiment (Figure 4) was carried out to study the action of heptaminol on calcium current and contraction. In this experiment, performed in sodium-free TEA-CH₃SO₃ Ringer, the preparation was depolarized to $E = -30$ mV by pulses of 2.5 s duration at a frequency of 1 min $^{-1}$; it developed a slow calcium inward current and contraction (see also Jacquemond & Rougier, 1988). When heptaminol (10^{-4} M) was added neither calcium current nor contraction were modified. Similar results were obtained in 4 other fibres.

These results indicate that the potentiating effect of heptaminol on tension cannot be due to modifications of the ionic fluxes occurring during the first steps of the process of excitation-contraction coupling.

Does heptaminol act via an internal alkalization?

Action of NH₄Cl It has been suggested that heptaminol exerts its positive inotropic effect by producing an internal alkalization (Berthiau *et al.*, 1989). The influence of internal alkalization on tension (see Fabiato & Fabiato, 1978) was tested in our experimental conditions by studying the action of NH₄Cl. It is well known that substituting NH₄Cl (20 mM)

Figure 4 Action of heptaminol on slow calcium current and tension. Heptaminol (10^{-4} M) had no action on slow calcium current and contraction. The preparation was stimulated at a frequency of 1 min $^{-1}$.

for NaCl induces a rapid alkalization of 0.2, 0.3 pH unit (Aickin & Thomas, 1977; Roos & Boron, 1981; Putnam *et al.*, 1986).

All the following experiments (Figures 5–8) were performed under voltage clamp conditions in the presence of TTX (10^{-7} M).

In Figure 5 the preparation was stimulated by voltage clamp depolarizing pulses (100 mV, 200 ms) at a frequency of 12 min $^{-1}$ inducing the phenomenon of fatigue. When NH₄Cl was introduced a marked and transient increase in tension developed. When returned to the control solution the tension decreased in two phases: an initial rapid phase which corresponded certainly to the sudden acidification due to NH₄Cl withdrawal (see Roos & Boron, 1981) followed by a slower one. Similar results were obtained in 5 other fibres.

These results indicate that an internal alkalization is able to increase the progressively declining tension observed during fatigue. But if the mechanism of NH₄Cl alkalization is well established (ROOS & BORON, 1981; LAZDUNSKI *et al.*, 1985 for a review), nothing is known concerning heptaminol; for example we have no evidence for a penetration of the molecule. In a series of experiments we found that tuamine, which corresponds to the amine group of heptaminol, decreased rather than increased tetanic tension during a train of repeated stimulation (not illustrated). Furthermore we observed that the presence of heptaminol (up to 10^{-2} M) in the 'internal' solution bathing cut fibres in the end pools of the double gap chamber did not modify the tension of the test gap fibre segment. So an intracellular alkalization by a penetration of the molecule as observed with NH₄Cl seems improbable.

Action on the Na/H antiport The possibility of an action of heptaminol on the Na/H antiport as mentioned in the introduction (Berthiau *et al.*, 1989) was investigated.

This hypothesis was attractive for several reasons. A stimulation of the Na/H antiport (1) increases the intracellular pH, a situation which, as observed under the action of NH₄Cl, is able to increase tension; (2) promotes the entry of sodium ions whose intracellular concentration is important for the control of contraction in cardiac (Lazdunski *et al.*, 1985) and in skeletal muscle (Caille *et al.*, 1985). Two kinds of experiments were carried out to test this hypothesis.

In Figure 6a the preparation was stimulated with the same protocol as in Figure 5 but in a sodium-free solution (TRIS substitute). The addition of heptaminol had no effect on the regularly declining tension. Similar observations were made on 5 other fibres. This result confirms and explains the absence of action of heptaminol on tension described in Figure 4 where calcium current and tension were studied in a sodium-free solution.

When the preparation was stimulated in the presence of an inhibitor of the Na/H exchange, addition of heptaminol

Figure 5 Action of NH₄Cl on tension during repeated stimulation in control solution (tetrodotoxin 10^{-7} M). The addition of NH₄Cl (20 mM) induced a marked increase in tension. Notice the sudden decrease in tension with NH₄Cl withdrawal. Same protocol of stimulation as in Figure 1a.

Figure 6 Action of heptaminol on tension during repeated stimulation in sodium-free or in amiloride-containing solution (tetrodotoxin 10^{-7} M). (a) In sodium-free solution (TRIS substitute) heptaminol (10^{-4} M) had no action on the declining tension. (b) In the presence of amiloride (10^{-3} M), heptaminol (10^{-4} M) had no action on tension. (a) and (b) were recorded from different fibres. Same protocol of stimulation as in Figure 1a.

(10^{-4} M) had no effect on tension; this was observed with amiloride (10^{-3} M) (Figure 6b) (4 preparations) and with EIPA (10^{-5} M) (not illustrated, 3 preparations).

It must be added that in our experimental conditions (performed in the presence of TTX) amiloride or EIPA had no action on the holding current and on the current during the depolarizing pulses. This conflicts with the recent work of Estrada & Sanchez (1991) who found a hyperpolarization as high as 12 mV in the presence of amiloride (10^{-3} M).

On the other hand the addition of NH_4Cl had always a potentiating action on tension in experiments performed in sodium-free amiloride (10^{-3} M) containing solution (Figure 7a, 3 preparations).

These results are in agreement with the hypothesis of a stimulation of the Na/H antiport by heptaminol. This conclusion is strengthened by the observation (3 preparations) that heptaminol was also active in sodium-free, lithium containing solution (Figure 7b), an experimental situation where the Na/H antiport is able to extrude H^+ (Aronson, 1985).

Discussion

Heptaminol has been shown to prevent fatigue of muscle and nervous activities (Chauchard & Mazoue, 1958; Rigal *et al.*, 1963). The cellular mechanisms underlying its action on skeletal muscle have been poorly studied. The results of our experiments show that heptaminol is able to prevent the decay or to increase the amplitude of tension during continuous repeated stimulation of single isolated twitch fibres.

Due to the presence of an alcohol group, heptaminol has general anaesthetic properties which can inhibit ionic conductances as shown by Haydon & Urban (1983) on squid giant axon; such an inhibition was observed at a concentration of 10^{-2} M. The absence of action of heptaminol at 10^{-4} M on the sodium, potassium and calcium currents is an indication that its effect on tension cannot be related to the general anaesthetic properties of the molecule.

An eventual action of heptaminol (10^{-4} M) on the intramembrane charge movements which are considered to play an

Figure 7 (a) Action of NH_4Cl on tension during repeated stimulation in sodium-free, amiloride-containing solution (tetrodotoxin (TTX) 10^{-7} M). In sodium-free (TRIS substitute) amiloride (10^{-3} M)-containing solution, addition of NH_4Cl (20 mM) induced a marked increase in tension. Same protocol of stimulation as in Figure 1(a). (b) Action of heptaminol on tension during repeated stimulation in lithium containing solution (TTX 10^{-7} M). Addition of heptaminol (10^{-4} M) delayed the decline in tension as observed in sodium-containing solution. Same protocol of stimulation as in Figure 1a.

important role in depolarization-contraction coupling (Schneider & Chandler, 1973) seems improbable. Indeed calcium channels as dihydropyridine receptors are considered to be the support of these charge movements (Rios & Brum, 1987); the fact that the calcium current is not modified by heptaminol is an indication that charge movements are certainly unaffected.

The absence of action of heptaminol during experiments with cut fibres is an indication that heptaminol does not act on some intracellular events implicated in the development of tension.

The possibility that heptaminol exerts its effect by producing an internal alkalization was tested by comparison with a NH_4Cl -induced alkalization. Both treatments had a positive inotropic effect but important differences were observed. The action of heptaminol was suppressed in experimental conditions where the Na/H exchange was inhibited, i.e. in sodium-free (TRIS substitute) and in amiloride containing solutions. The action of NH_4Cl was always observed in the same experimental situations. Furthermore, heptaminol exerted its action in sodium-free, lithium containing solution.

These results have several interesting meanings: (1) amiloride and its derivative EIPA cannot be considered in our experimental conditions as direct inhibitors of the sarcoplasmic reticulum calcium release as suggested by Nasri-Sebdani *et al.* (1989); (2) heptaminol is not able to produce directly an internal alkalization as does NH_4Cl ; (3) the fact that heptaminol exerts its action in lithium as well as in sodium solution indicates that the Na/Ca exchange (which is not working in lithium solution) cannot be implicated; on the other hand this strengthened considerably the implication of the Na/H exchange (which is working in lithium solution).

In conclusion the positive inotropic effect of heptaminol on skeletal muscle can be explained by an internal alkalization due to a stimulation of the Na/H exchange system. This is in agreement with the results of Berthiau *et al.* (1989) on cardiac muscle. The mechanism(s) of heptaminol Na/H antiport stimulation remains to be studied; an alteration of the apparent affinity of the exchange for internal H^+ can be suggested as postulated by Moolenaar *et al.* (1983) for the action of growth factors. Whatever the mechanisms, these experimental results show that the Na/H exchanger plays an important role during the appearance of fatigue; any situation which stimu-

lates this exchange can induce (1) an intracellular alkalization which can increase tension through an action on contractile proteins (Fabiato & Fabiato, 1978), (2) an influx of sodium which can increase tension by an action on the sarcoplasmic reticulum calcium release (Caille *et al.*, 1978; Allard & Rougier, 1989b).

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