

## Effects of Na-Octanoate on Potassium Contractures in Normal and Denervated Frog Tonic Fibres\*

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**Abstract.** In frog twitch muscle fibres, Na-octanoate ( $\text{NaC}_8$ ) shifted the relation between potassium induced tension and membrane potential to the right. The present study has been carried out to investigate the effect of this fatty acid on frog tonic fibres. Potassium contractures measured on bundles of 30—40 fibres of ileofibularis muscles were less decreased by  $\text{NaC}_8$  (2.5—10 mmol/l) than those of twitch fibre bundles. In denervated muscles the sensitivity to  $\text{NaC}_8$  was increased, probably due to the development of sodium channels in the membranes. Experiments with mixed fibre bundles also showed a lower influence of  $\text{NaC}_8$  on potassium contracture of tonic fibres. On the other hand, tonic fibres showed a lower threshold of the potassium induced tension as well as a lower  $\text{K}^+$  concentration for maximal activation. This lower threshold was further lowered by  $\text{NaC}_8$ , corresponding to a shift of the relation between potassium concentration and tension to the left. The membrane resting potentials were  $-58 \pm 9$  mV in tonic fibres and  $-83 \pm 5$  mV in twitch fibres. Five mmol/l  $\text{NaC}_8$  only induced depolarization of the membrane of tonic fibres. This depolarization (by about 20 mV) may be responsible for the threshold shift to lower  $\text{K}^+$  concentration in  $\text{NaC}_8$ -exposed tonic fibres. In addition to the effects of  $\text{NaC}_8$  on sodium channels, interactions with  $\text{Ca}^{2+}$  binding sites are discussed.

**Key words:** Tonic muscle fibre — Denervation — Na-octanoate — Potassium contracture — Threshold shift — Membrane resting potential

### Introduction

Na-octanoate ( $\text{NaC}_8$ ) and other fatty acids decrease twitch and tetanic contractions of frog and rat skeletal muscles and the contractility of heart muscles of various mammals (Kössler et al. 1976, 1980; Caffier and Pfeiffer 1977; Caffier et al. 1982; Kössler and Borovikow 1983; Opie 1970; Vik-Mo and Mjos 1981; Corr et

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al. 1982). In twitch (phasic) fibres of frog ileofibularis muscles,  $\text{NaC}_8$  depresses the amplitude of potassium contracture and shifts the relation between potassium concentration (or membrane potential) and tension to the right. As a result  $\text{NaC}_8$  renders the contractile activation process less sensitive to membrane depolarization by  $\text{K}^+$  and deteriorates the excitation-contraction (E-C) coupling. This inhibition can be compensated for by small doses of caffeine (Caffier et al. 1982).

Frog tonic fibres are devoid of the action potential mechanism (Kuffler and Vaughan Williams 1953; Nasledov 1981). In  $\text{K}^+$  rich solution these fibres show a slower tension development than do twitch fibres, and maintain contraction for a relatively long period of time (Nasledov 1981; Nasledov et al. 1966; Lännergren 1967). However, after denervation tonic fibres are able to develop the action potential mechanism, and their responses in high  $\text{K}^+$  solution become less stable (Elul et al. 1968; Nasledov and Thesleff 1974; Lapshina and Nasledov 1978; Schalow and Schmidt 1977; Schmidt 1980).

Recent discussions have suggested that the presence of the E-C coupling mechanism is unlikely in amphibian twitch and tonic fibres (see Godt et al. 1984). Obviously, some differences exist in the activation between these two fibre types, and these differences become diminished by denervation. Since octanoate and other fatty acids may interact with E-C coupling via alterations of ionic channels and/or  $\text{Ca}^{2+}$  binding sites (Caffier et al. 1982) it seems of interest to establish whether Na-octanoate can influence tonic fibres to the same extent as it does with twitch fibres, and whether denervation would be able to modify the effects of octanoate.

## Materials and Methods

Experiments were performed with tonic fibre bundles (30–40 fibres) isolated from ileofibularis muscle of the frog *Rana temporaria*, using a stereomicroscope. The fibre bundles were mounted horizontally in a Perspex chamber filled with Ringer solution (mmol/l: NaCl 111; KCl 2.5;  $\text{CaCl}_2$  1.8;  $\text{NaHCO}_3$  2.4; pH 7.2). One end of the bundle was fixed to the chamber bottom, the other one to a force transducer (mechanotron tube 6MX 2B). To induce potassium contractures NaCl was replaced with isoosmolar concentrations of KCl. Usually, a concentration of 80 mmol/l  $\text{K}^+$  was applied. The exchange procedure caused small artifacts on the running records. The tension developed in high  $\text{K}^+$  solution was recorded using a paper recorder or an oscillograph (C 1–18). After a short exposure to high  $\text{K}^+$  solution, the bundles were perfused with normal Ringer solution for at least 10 min to induce repeated contractures by high  $\text{K}^+$ . In this way, the effect of  $\text{NaC}_8$  could be tested on the same fibre bundle which was used in the control test. Octanoate was added to the Ringer solution but not to the high  $\text{K}^+$  solution, since preliminary studies on twitch fibres with octanoate present in both solutions failed to show any difference.

Denervation of muscles was performed by cutting off the right sciatic nerve under ether anaesthesia. About 5 mm of the cut nerve were removed (near the ileococcygeal junction). The skin was closed with two stitches. Following this procedure, frogs were kept at low temperature until the experiments were performed 7 or 28 days later. The data shown on the Figures are representative of a series of at least three repeated experiments carried out during May–July at temperatures of 22 to 26 °C.

## Results

### Normal muscles

Contractures in tonic fibre bundles. Contractures induced by 80 mmol/l  $K^+$  were repeated two or three times in Ringer solution. When identical tension developed at each occasion, the bundles were exposed to a solution containing  $NaC_8$  for 10 min. The lowest concentration (2.5 mmol/l) had no distinct effect on the tension amplitude, but a small increase in the resting tension and a slight modification of the relaxation phase seemed to occur. Five mmol/l  $NaC_8$  induced both, a pronounced increase in the resting tension during octanoate exposure and a depression of the following potassium contracture by about 10–20% (Fig. 1). The effects were reversible in Ringer solution. After 10 mmol/l  $NaC_8$  the tension amplitude decreased by 20–30%. In twitch fibre bundles, the corresponding depression was 40 and 60% in 5 or 10 mmol/l  $NaC_8$ , respectively (Caffier et al. 1982).

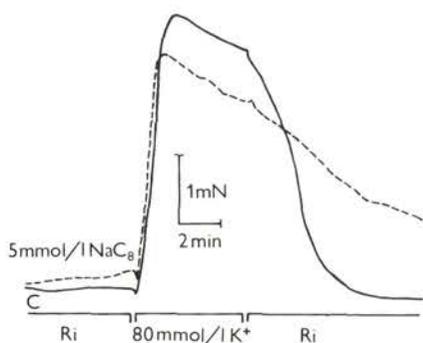
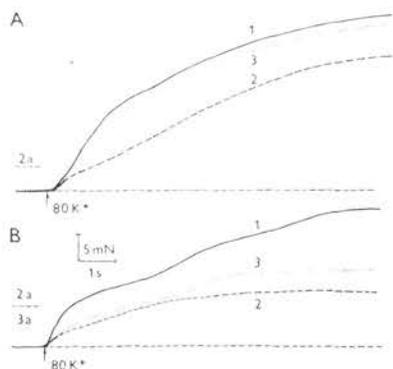


Fig. 1. Effect of 5 mmol/l Na-octanoate ( $NaC_8$ , interrupted line) on the potassium induced contracture (80 mmol/l  $K^+$ ) in a frog tonic fibre bundle. C: control.

Membrane resting potential. Frog tonic fibres have lower membrane potentials than do twitch fibres. In Ringer solution a potential of about  $-50$  mV was reported (Kuffler and Vaughan Williams 1953; Kiessling 1960; Nasledov 1981; Godt et al. 1984). The lower membrane potential was explained by a high input resistance of tonic fibres leading to leakage currents during electrode impalements (Stefani and Steinbach 1969; Morgan and Proske 1984). In the present experiments, mean potentials of  $-58 \pm 9$  mV were measured (5 bundles 10 fibres each). Fibres of preparations showing membrane potentials of 80 to 90 mV were identified as being twitch fibres. Five mmol/l  $NaC_8$  which had no effect on the membrane resting potential of twitch fibres, induced membrane depolarization of tonic fibres by about 20 mV. During 10–20 min exposure to this concentration the membrane potentials dropped to a mean level of  $-37 \pm 6$  mV (3 bundles 10 fibres each). Following octanoate washout repolarization was observed.

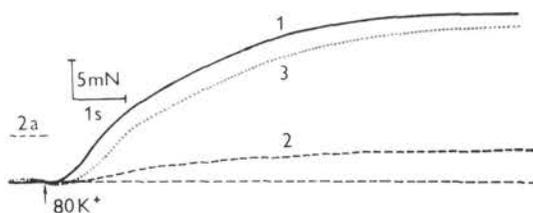
### Denervated muscles

The membrane resting potential of denervated tonic fibres was found not to differ from that of normal muscles (Lapshina and Nasledov 1981). Potentials of  $-56$  to  $-58$  mV were measured. In tonic bundles of denervated muscles,  $2.5$  mmol/l  $\text{NaC}_8$  induced a stronger spontaneous increase of the resting tension (fatty acid contracture) than in bundles of normal muscles, and the subsequent potassium contracture was lower than the preceding one without octanoate (Fig. 2A, B). Octanoate-induced changes in tension are represented in Fig. 2 by the interrupted lines of the superimposed oscillographic records just before the addition of potassium (arrows). The tension increase was compensated for electrically to allow a comparison between the contractures induced by high  $\text{K}^+$  solution before and after octanoate exposure. The depression caused by  $2.5$  mmol/l  $\text{NaC}_8$  was about 20 % in control muscles and about 60 % in denervated muscles. The lower  $\text{K}^+$ -induced tension summed with the octanoate-induced contracture (Fig. 2B, line 2a), give a total tension increase by 70 % of the control value.



**Fig. 2.** Superimposed potassium contractures ( $80$  mmol/l  $\text{K}^+$ ) from oscillographic records of tonic fibre bundles before (1), and immediately after 10 min exposure to  $2.5$  mmol/l Na-octanoate (2), and after 15 min in Ringer (3); 2a, 3a: octanoate induced tension increase before the addition of  $\text{K}^+$ . A: contralateral muscle. B: denervated muscle; 7th day.

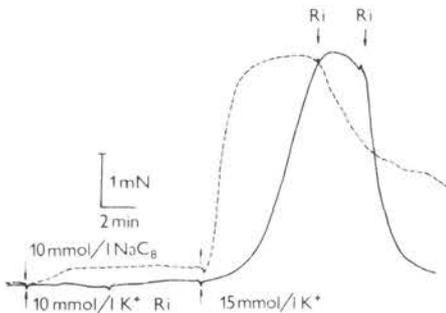
Exposure to  $5$  mmol/l  $\text{NaC}_8$  also induced an increase in the resting tension and depressed the subsequent potassium contracture by 80 % (Fig. 3, curve 2). In Ringer solution, the effect was reversible (curve 3).



**Fig. 3.** Superimposed potassium contractures of a tonic fibre bundle of a 4 weeks denervated muscle from oscillographic records before (1), and immediately after 10 min exposure to  $5$  mmol/l Na-octanoate (2), and after 15 min in Ringer (3); 2a: octanoate induced tension before the addition of  $\text{K}^+$ .

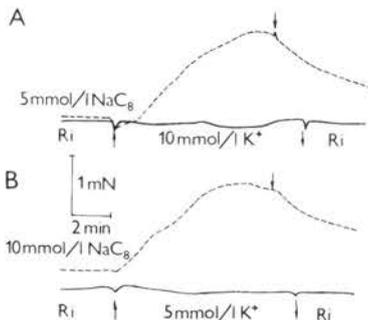
*Octanoate induced threshold shift*

Octanoate rendered twitch fibres less sensitive to  $K^+$  depolarization. Tonic fibres showed inverse responses. One characteristic of the higher sensitivity of tonic fibres was an increase in the resting tension in 5 and 10 mmol/l  $NaC_8$  resulting from slow depolarization in these solutions. Another aspect was the earlier development of tension in tonic fibres pretreated with octanoate, when 15 mmol/l of  $K^+$  were added, a concentration near the threshold for these fibres (Fig. 4). At this low  $K^+$



**Fig. 4.** Effects of 10 mmol/l and 15 mmol/l  $K^+$  on normal tonic fibre bundle (full line, 10 mmol/l  $K^+$  induced no contracture). Following 10 min pretreatment in 10 mmol/l  $Na$ -octanoate (interrupted line, without  $K^+$ ); the addition of 10 mmol/l  $Na$ -octanoate accelerated the tension development. Arrows indicate the addition and the removal of  $K^+$  and  $NaC_8$ , respectively. Artifacts were caused by solution exchange.

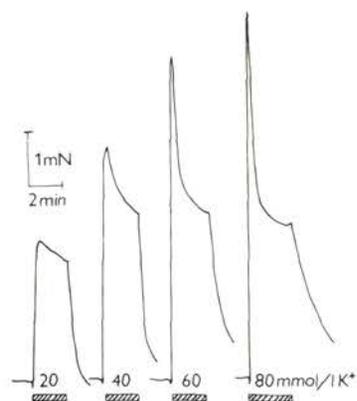
concentration, normal tonic fibres needed 7–8 min to develop maximum tension;  $NaC_8$ -pretreated bundles reached the same tension within 4 min. The concentration of 10 mmol/l  $K^+$  was subthreshold and no tension development could be observed during an exposure over more than 20 min. Following pretreatment of the tonic bundle with 5 mmol/l  $NaC_8$  (10 min) the subthreshold potassium concentration of 10 mmol/l induced a distinct contracture (Fig. 5A). Moreover, a 10 min exposure to 10 mmol/l  $NaC_8$  enabled the fibres to produce a contracture following the addition of as little as 5 mmol/l  $K^+$  (Fig. 5B). This clear threshold shift was mediated by the cooperative effect of the fatty acid and potassium.



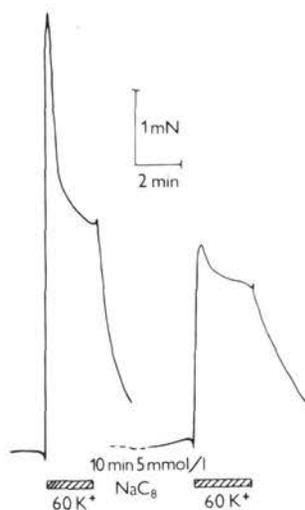
**Fig. 5.**  $Na$ -octanoate induced threshold shifts of potassium contractures of normal tonic fibres. The arrows indicate the addition and removal of  $K^+$ ; solution exchange was accompanied by artifacts. **A:** No tension development in 10 mmol/l  $K^+$  during 10 min (full line). Following 10 min pretreatment in 5 mmol/l  $NaC_8$ , 10 mmol/l  $K^+$  induced contracture in the same tonic fibre bundle (interrupted line). **B:** No tension development in 5 mmol/l  $K^+$  (full line). Following 10 min pretreatment in 10 mmol/l  $NaC_8$ , as little as 5 mmol/l  $K^+$  induced contracture (interrupted line).

### Mixed fibre bundles

For these studies, bundles containing both types of muscle fibres were prepared. They could be identified by biphasic relaxation in 80 mmol/l  $K^+$ . The fast tension drop was attributed to twitch fibres, and the slow one to tonic fibres. Actually, these two fibre types also responded to different threshold concentrations (Fig. 6):



**Fig. 6.** Effects of 20; 40; 60; and 80 mmol/l  $K^+$  on mixed fibre bundles of frog ileofibularis muscle. Exposure to  $K^+$  is marked by hatched areas under the curves. Slow relaxation sectors represent tonic fibres, fast relaxation slopes are due to phasic fibres.



**Fig. 7.** Potassium contractures (60 mmol/l  $K^+$ ) of a mixed fibre bundle of frog ileofibularis muscle without (left) and with 10 min pretreatment in 5 mmol/l  $NaC_8$  (right). Exposure to  $K^+$  are indicated by the hatched areas under the curves.

20 mmol/l  $K^+$  activated tonic fibres only; 40 mmol/l  $K^+$  increased the tension of tonic fibres and activated some phasic fibres as well, 60 and 80 mmol/l  $K^+$  induced almost the same maximal tension in both fibre types. The tension development was very similar in 40, 60, and 80 mmol/l  $K^+$  with tonic fibres. This experiment suggested that in tonic fibres, both the threshold potassium concentration and

saturation (maximum tension) were lower than the corresponding values for twitch fibres. This was more exactly shown in earlier experiments on isolated single fibres (Lännergren 1967; Nasledov et al. 1966). Finally, a different sensitivity to Na-octanoate was shown on mixed fibre bundles (Fig. 7). Following a 10 min exposure to 5 mmol/l NaC<sub>8</sub>, in 60 mmol/l K<sup>+</sup> solution the tension development was completely abolished in twitch fibres while the tension amplitude of tonic fibres was scarcely reduced. These findings confirmed the suggested stronger effect of octanoate on the potassium-induced tension development in twitch fibres.

### Discussion

Fibre bundles of skeletal muscles are a useful tool in muscle research (see, e. g. Caffier et al. 1982; Gilly and Hui 1980a; Godt et al. 1984; Hayatsu et al. 1981). They are small enough to permit a sufficient diffusion of metabolic substances or drugs, and their mechanical responses to stimulation can be recorded much more easily than those of single fibres. Low concentrations of free fatty acids influence mechanical and electrical properties of heart and skeletal muscles (Opie 1970; Caffier et al. 1982; Corr et al. 1982; Katz et al. 1982), but the mechanisms mediating these effects are not known. Inhibition of processes of Ca<sup>2+</sup> uptake and release as well as a blockade of ionic channels in the membrane have been professed as explanation (Kössler et al. 1980; Caffier and Küchler 1980; Caffier et al. 1980). If sodium channels were altered, fatty acids should induce weaker effects on membranes lacking sodium channels. Frog tonic fibres seemed to be a suitable preparation since the absence of voltage-dependent sodium channels in their membranes has been presumed (Gilly and Hui 1980b). These fibres were not able to respond to a single electrical stimulus but developed pronounced contractures in high K<sup>+</sup> solutions. Following denervation, sodium channels were formed which had the same ionic selectivity as did Na<sup>+</sup> channels of other cell membranes under comparable methodical conditions (Zacharová et al. 1983).

Comparing the results obtained on normal tonic fibres with those obtained on phasic fibres, it can be concluded that the effect of octanoate on potassium contractures is smaller in tonic fibres. On the other hand, effect of octanoate on denervated tonic fibres was increased (Fig. 2). These findings suggest that membranes containing Na<sup>+</sup> channels possible are more sensitive to fatty acids. Indeed, the sodium conductances ( $G_{Na}$ ) of the two fibre types are quite different: 50 mS/cm<sup>2</sup> in twitch fibres and 0.02 mS/cm<sup>2</sup> in tonic fibres, while there are only small differences in  $G_K$  (Gilly and Hui 1980b, c). In tonic fibres, identical Na<sup>+</sup> channels formed in response to denervation may be a result of the expression of genes responsible for the sodium channel protein; these genes are suppressed in intact nerves (Nasledov and Thesleff 1974; Zacharová et al. 1983). In view of this suggestion the presence of sodium channels may be an essential factor for a stronger octanoate-induced tension decrease in twitch and denervated tonic fibres.

In tonic fibres, especially those of denervated muscles, low concentrations of octanoate induced an increased resting tension. Such fatty-acid-induced contractures have never been observed on twitch fibres exposed to similar concentrations of  $\text{NaC}_8$  (Kössler and Kuchler 1977). This difference may be due to the mode of E-C coupling which has been related to the proportion of tubular and sarcoplasmic membrane structures (see Morgan and Proske 1984). The membrane resting potential of frog twitch fibres is about  $-85$  mV. The lower values of tonic fibres (about  $-60$  mV) were closer to the threshold for mechanical activation. As  $5$  mmol/l octanoate additionally decreased the membrane potential by about  $20$  mV, activation of the tension development seems to be possible for this contracture. Also, this fatty-acid-induced depolarization may co-operate with the subthreshold potassium depolarization and consequently reach the threshold for contracture by  $\text{K}^+$  concentrations as low as one third of those needed in the absence of octanoate.

Contrary to the octanoate-induced shift to the right of the relation between peak tension and potassium concentration in twitch fibres (Caffier et al. 1982), the membrane of tonic fibres becomes more sensitive, i. e. the values in the abscissa are shifted to the left, corresponding to a lower threshold for  $\text{K}^+$  contractures. In recent works it has been suggested that the thresholds for potassium contractures are different for the two types of frog muscle fibres: about  $15$  mmol/l  $\text{K}^+$  for tonic fibres (Hayatsu et al. 1981) and  $20$  mmol/l  $\text{K}^+$  for twitch fibres (Hodgkin and Horowicz 1960; Kirby et al. 1973; Lüttgau and Spiecker 1979). Similar threshold concentrations are suggested by the present observations on mixed fibre bundles. Differences also exist in  $\text{K}^+$  concentrations needed for maximum peak tension (saturation of contracture).

In earlier reports octanoate was proposed to interact with  $\text{Na}^+$  and  $\text{K}^+$  channels of twitch fibres as well as with E-C coupling mechanisms and  $\text{Ca}^{2+}$  binding sites (Caffier et al. 1980, 1982; Kössler and Kuchler 1977). These suggestions may also hold for tonic fibres; however in these muscle cells, processes such as  $\text{Ca}^{2+}$  release, cross-bridge turnover,  $\text{Ca}^{2+}$  dislocation from troponin,  $\text{Ca}^{2+}$  uptake by the sarcoplasmic reticulum, are supposed to be much slower than the respective processes in twitch fibres (Gilly and Hui 1980a). These differences may modify the octanoate effect. Which of the various mechanisms is altered by the fatty acid has to be revealed by further experiments.

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