# Histidine and carnosine reduce muscle fatigue in *Xenopus laevis*

Lesley Whitaker and G.N. Louw
Zoology Department, University of Cape Town, Rondebosch

The inclusion of either histidine or carnosine in a frog Ringer's perfusate at a concentration of 20 mmol/dm$^3$ reduced fatigue in the gastrocnemius muscle of *Xenopus laevis* significantly. Histidine was superior to carnosine in this respect. The evidence also suggests that muscles perfused with histidine exhibit superior recovery, accumulate less lactic acid and lose significantly less $K^+$ from the intracellular fluid. The implications of these findings in future research on capture myopathy are discussed.
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Die insluiting van histidien of karnosien by 'n Ringer-perfusaat teen 'n konsentrasie van 20 mmol/dm$^3$ het spieruitputting in die gastrocnemius-spier van *Xenopus laevis* betekenisvol verminder. Histidien was in dié opsig doeltreffender as karnosien. Die data toon ook dat spiere wat met histidien deurgespoel is, geneig het om vinniger te herstel, minder melksuur op te hoop en betekenisvol minder $K^+$ uit die intrasellulêre vloeistowwe te verloor. Die implikasies van hierdie bevindings in toekomstige navorsing op vangmiopatie word bespreek.
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**Lesley Whitaker* and G.N. Louw**
Zoology Department, University of Cape Town, Rondebosch, 7700 Republic of South Africa
*To whom correspondence should be addressed

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Although it is now general knowledge that both histidine and carnosine fulfil an important buffering function in muscle cells (Long 1961), their ability to reduce muscle fatigue has not yet been quantified. Therefore the purpose of this study was to examine the effects of these naturally-occurring cell buffers on muscle fatigue, lactic acid accumulation, pH and potassium efflux in the gastrocnemius muscle of *Xenopus laevis*. The motivation for the study was provided by our interest in finding a suitable buffer for treating the acute muscle acidosis which develops in capture myopathy.

## Procedure

We made use of the frog gastrocnemius preparation. The animals were killed by double pithing and placed ventral side up in a wax-filled dissecting dish. The distal end of the gastrocnemius on the left side was dissected free, before attaching it by means of a cotton thread to an isotonic lever, connected to a transducer and polygraph recorder. This procedure left the nervous and blood supply intact at the proximal end of the muscle. The ipsilateral sciatic nerve was also dissected free and attached to two silver electrodes, connected to a stimulus generator.

To perfuse the muscle a catheter was inserted into the dorsal aorta and the perfusate was administered at a pressure of 35 – 45 mm Hg (Shelton & Jones 1968). A cannula, inserted in the posterior vena cava, allowed collection of the effluent perfusate after it had circulated through both hind limbs. The external surface of the gastrocnemius, attached to the isotonic lever, was also irrigated continuously with the relevant perfusate throughout the experiment. The perfusate used in the control experiments (Frog Ringer's) consisted of 6,50 g NaCl $l^{-1}$; 0,14 g KCl $l^{-1}$; 0,12 g $CaCl_2$ $l^{-1}$ and 0,20 g $NaHCO_3$ $l^{-1}$ (Hoar & Hickman 1975). When testing the effects of either histidine or carnosine they were added individually, in chemically pure form (Sigma), to the Ringer's solution to obtain a final concentration of 20 mmol/dm$^3$ of the relevant buffer. The relative efficacy of L and DL-histidine was examined in separate experiments and, when measuring the effects of the buffers on the efflux of $K^+$, this ion was omitted from the perfusate in a separate experiment.

To quantify fatigue in the gastrocnemius, the muscle was first perfused for 30 min while at rest, before stimulating the sciatic nerve continually at intervals of 2 s with a voltage of 0,5 V. The amplitude of the resulting muscle contractions was then continuously recorded on the polygraph until exhaustion occurred, whereupon stimulation was terminated. The muscle was then allowed a 5 min recovery period and again stimulated

to exhaustion. The alternate resting and stimulating of the muscle was repeated six times after the first initial exhaustion. At the end of each trial the gastrocnemius was dissected out, weighed, homogenized in 20 ml of the control perfusate and the pH was measured. The homogenate was then centrifuged in a clinical centrifuge and the concentration of lactic acid in the supernatant was enzymatically determined using lactate dehydrogenase (Boehringer Mannheim, UV method). Lactic acid values were expressed in terms of the original wet muscle mass.

In the separate experiment designed to compare the effects of the control perfusate and the perfusate containing L-histidine upon $K^+$ efflux, the effluent perfusate from the posterior vena cava was collected separately for 10 min before muscle stimulation, for 10 min during stimulation and during a 5 min rest period immediately following stimulation. This procedure was repeated three times for each preparation. Concentration of $K^+$ in the effluent was determined by a standard flame photometric technique (Instrumentation Lab. IL243).

Statistical analyses consisted of comparing each treatment separately using the appropriate Student's *t* test (Zar 1974). Unless otherwise stated each treatment was replicated eight times.

## Results

The results illustrated in Figure 1 show highly significant differences in the length of time taken for the muscles to reach exhaustion in the three different treatments ($p < 0{,}01$). In the case of the control perfusate, exhaustion was reached after a mean interval of 12 min, while the carnosine and histidine treatments extended this period to 40,1 and 71,0 min respectively. The naturally occurring amino acid L-histidine was more effective than DL-histidine while carnosine gave inferior and more erratic results than both isomers of histidine.

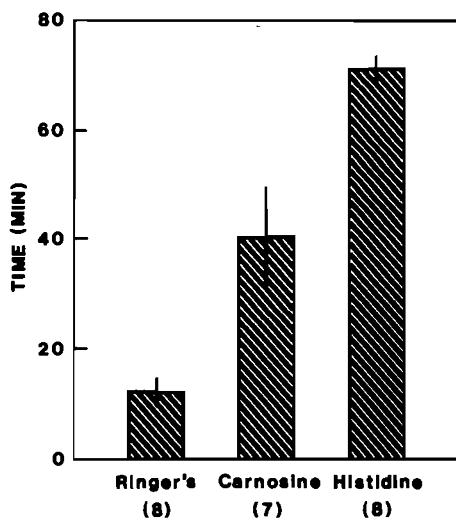


**Figure 1** Length of time taken for muscles to reach exhaustion in the three treatments. Vertical bars represent standard errors, with number of replications in parentheses.

The amplitude of contraction declined rapidly with time in all treatment groups but much more rapidly in the case of the control perfusate than with the perfusates containing buffers. For example, after 12 min the amplitude of contraction with the control perfusate had declined to 1,26 ± 0,67% (mean ± S.E.) of the original amplitude at the start of the experiment, whereas in the case of the carnosine and histidine treatments the amplitude of contraction had declined respectively to 13,92 ± 4,46% and 15,25 ± 4,05% of the original amplitude. These differences, after arcsine conversion, were statistically significant ($p < 0{,}01$). A comparison of the rate of decline in amplitude of contraction between the L-histidine and the control treatment appears in Figure 2. This figure also illustrates the much shorter period required to stimulate the muscle receiving the control perfusate to exhaustion.

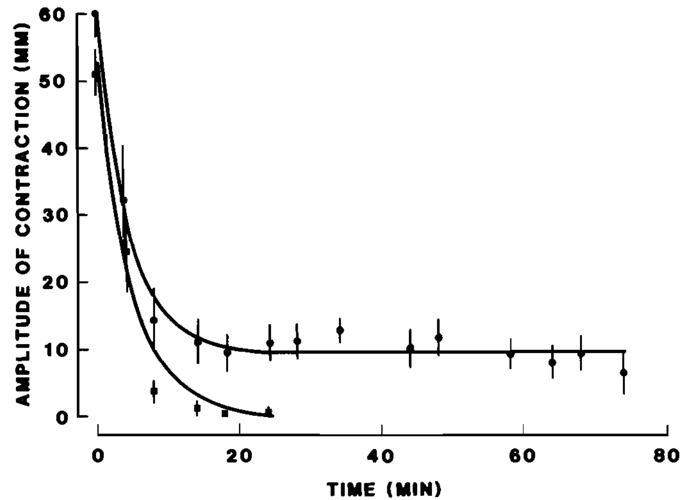


**Figure 2** Rate of decline in amplitude of muscle contraction when perfusing with Ringer's (■) and Ringer's plus histidine (●). Vertical bars represent standard errors.

The recovery of the muscles during the 5 min rest periods, between the seven periods of stimulation to exhaustion, also differed between treatments. This was evaluated on the basis of the amplitude of contraction after the rest period and again the histidine treatment provided the highest values, followed by carnosine, with the control perfusate giving the lowest values and the steepest rate of decline in amplitude during the entire experiment (Figure 3). These differences were, however, not

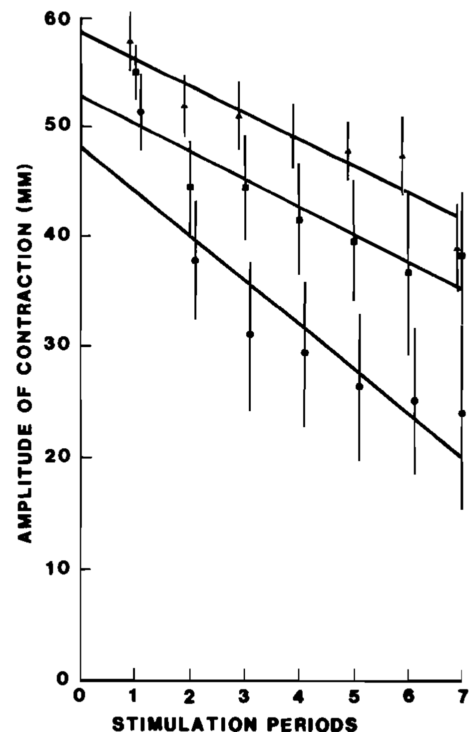


**Figure 3** Amplitude of muscle contraction immediately after a 5 min rest period. Prior to the seven rest periods the muscles were stimulated to exhaustion. Ringer's perfusate (●), Ringer's plus histidine (▲), Ringer's plus carnosine (■). Vertical bars represent standard errors.

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statistically significant owing to the high coefficient of variation in the data and probably because of the different contraction periods preceding exhaustion in the different treatment groups.

The pH values obtained from the muscle homogenates, after the seventh period of stimulation, did not differ significantly ($p > 0,05$), but it should be remembered that the contraction time and work output of the muscles perfused by the buffers were far greater than in the case of the muscles receiving the control perfusate. The comparison is therefore not strictly valid. Nevertheless, in spite of the differences in contraction time, the lactic acid concentration in the muscles perfused by L-histidine tended to be lower than those receiving the control treatment (Table 1). These differences, however, just failed to reach statistical significance ($p > 0,05$), but again the same arguments used for the pH data apply.

**Table 1** Effect of inclusion of histidine and carnosine in Ringer's muscle perfusates on lactic acid accumulation in and $K^+$ efflux from frog muscle (means ± S.E.)

| | Treatment | | |
|---|---|---|---|
| | Carnosine | Histidine | Ringer's only |
| Lactic acid content (expressed as mg $l^{-1}$ supernatant per g wet muscle extracted) | 48,3 ± 7,1 ($n$ = 7) | 35,5 ± 5,0 ($n$ = 8) | 51,8 ± 13,6 ($n$ = 8) |
| Potassium ion concentration in effluent (mmol/$dm^3$) | – | 0,29 ± 0,02 | 0,46 ± 0,03 |
| Number of trials | | 26 | 27 |

In the separate experiment designed to study the effect of the buffers on $K^+$ efflux, the complication of variable work output did not apply as the contraction period was the same for all treatments. In this case the inclusion of histidine in the perfusate reduced the $K^+$ concentration significantly ($p < 0,01$) when compared with the control treatment (Table 1).

## Discussion and Conclusions

The inclusion of the naturally occurring muscle cell buffers, histidine and carnosine, in perfusates reduced fatigue in the frog gastrocnemius muscle dramatically. There is a marked and highly significant increase in contraction time to exhaustion when the buffers are included in the perfusate. The rate of reduction in amplitude of contraction during continual stimulation is also slower in muscles perfused with the buffers. A strong tendency was recorded for lower accumulations of lactic acid and superior recovery from exhaustion in muscles receiving the buffered perfusates than those receiving the control perfusate. The histidine treatment also reduced the efflux of $K^+$ from the contracting muscle significantly, and L-histidine appeared to be superior to carnosine in respect to all the above effects.

In view of these encouraging results and the apparent importance of muscle acidosis in the development of capture myopathy (Harthoorn, van der Walt & Young 1974) some speculation on the possible use of these buffers for the treatment of this condition seems justified. For example Harthoorn *et al.* (1974), having established the importance of muscle acidosis in the entrainment of the myopathy syndrome, advocated the use of $NaHCO_3$ infusions for the treatment of the condition. Later Gericke, Hofmeyr & Louw (1978) completed a detailed study of the physiological responses and changes in blood chemistry of capture-stressed springbok. When springbok are chased beyond their normal limits of endurance, which are surprisingly restricted, the following sequence of responses develops. As expected, there is first a marked increase in respiration and heart rate, together with a swift rise in body temperature. The oxidative capacity of the muscles is soon exceeded and the resultant increase in lactic acid and lowering of the pH appears to affect the integrity of the muscle cell membranes. As a consequence there is a dramatic rise in plasma levels of various muscle enzymes, particularly CPK and LDH. Simultaneously an efflux of $K^+$ occurs from the muscle cells and the resulting hyperkalaemia may be the ultimate cause of death by disrupting normal membrane function of the cardiac muscle. Similar results were recorded for chased zebras (Hofmeyr, Louw & du Preez 1973) and in birds captured in mist nets (Henschel & Louw 1978).

Nevertheless, it should be remembered that in the present investigation the animals were pre-treated with the buffers and it remains an open question if the buffers would be effective after this complex syndrome has been entrained. In addition, the present study was carried out on an ectothermic animal at 21 °C whereas hyperthermia (>42 °C) is an important complicating factor in capture myopathy in the field.

We are unable to provide an explanation at this stage for the exact mode of action of the buffers in reducing muscle fatigue. It is, however, felt that the results are sufficiently encouraging to suggest further research on the basic cell biology involved as well as on the possible clinical use of these compounds for the treatment of capture myopathy.

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