Arab Gulf J.Scient. Res., **12** (1), pp.157 - 170(1994)

Effect of Acute Hypotension on the Mechanical Response of the Rat C-Polymodal *Nociceptors*

Jumah Shakhanbeh

Department of Biology, Faculty of Science, Mu'tah University, P.O.Box 7, Karak, Jordan

ABSTRACT. Mechanical response of the rat C-Polymodal nociceptors (C-PMNs) to repeated controlled forces (10-100 mN) was studied before, during and after acute severe reducion in the blood pressure (hypotension). Von Frey pressure thresholds of these units were not affected by the hypotensive conditons. However, the mechanical response of the C-PMN units to repeated controlled mechanical forces was markedly decreased in 9 units (out of 10 units) during and after hypotension compared to their normal response before hypotension. The reduction in the excitability of the rat C-PMNs to strong mechanical stimulation under the conditions of acute hypotension is probably due to the decrease in the amount of oxygen supply with the subsequent accumulation of metabolites nearby their nerve terminals.

The mechano-heat sensitive C-fiber receptors group (C-polymodal nociceptors) is the largest of sensory fiber receptors which form 50-75% of the total afferent unmyelinated C-fibers in cutaneous nerves (Lynn and Carpenter 1982, Chad *et al.* 1983, Fleischer *et al.* 1983, Lynn 1984, Shea and Perl 1985 and Carter and Lisney 1987). This group of nerve fibers respond to a variety of moderately intense and potentially damaging thermal, mechanical and chemical stimuli (Torebjork 1974, Croze *et al.* 1976, Beitel and Dubner 1976, Shea and Perl 1985 and Campbell *et al.* 1988). The normal discharge pattern of these units in response to various stimuli has been extensively studied during repeated noxious heat stimuli (Handwerker and Neher 1976, Lynn 1979, Fitzgerald 1979, Lynn and Carpenter 1982, Lamotte *et al.* 1983, Campbell and Meyer 1983 and Adriaensen *et al.* 1984), mechanical forces (Shakhanbeh and Lynn 1992) and chemical treatment (Kenins 1982, Szolcsanyi 1987, Szolcsanyi *et al.* 1988, He *et al.* 1988, Lang *et al.* 1990, Belmonte *et al.* 1991 and Pozo *et al.*1992). Moreover, under certain abnormal experimentally induced pathological

conditions, such as acute inflammation, the nociceptive primary afferent function (Russell *et al.* 1987, Kocher *et al.* 1987 and Kirchhoff *et al.* 1988) and efferent response (Shakhanbeh unpublished data) of the C-PMNs have been markedly enhanced. The effect of acute reduction in blood pressure has not, however, so far, been studied on the mechanical response of the C-fiber nociceptors. The aim of this study was to examine the excitability of the C-PMNs in the rat skin to repeated controlled mechanical forces under the conditions of acute severe reduction in the blood pressure.

Materials and Methods

Animal preparation

Male Sprague-Dawley rats weighing 200-550 g, were initially anesthetized by the intraperitoneal injection of 50 mg/kg of Sodium pentobarbitone (Sagatal, May and Baker). To maintain deep level of anesthesia, additional doses (20 mg^{-1} kg/ hr) were intravenously administered through the cannulated external jugular vein. A tracheal cannula was inserted, and rectal temperature was kept around 37°C by a heating pad under the animal. Arterial blood pressure was monitored continuously from a cannula inserted in the left common carotid artery.

Single unit isolation and stimulation

The saphenous nerve was exposed, cut in the upper thigh, and covered with a pool of liquid paraffin made from skin flaps stitched around a brass ring. For isolation of single units, a pair of stimulating platinum electrodes were placed under the nerve at a point just above the knee. Electrical stimulation for the C-fibers was done at 1 to 5 milliamperes (mA), 0.5 millisecond (ms) pulse duration and at 1 Hz frequency. Recording was made from fine filaments dissected from the proximal cut end of the nerve using watchmakers forceps and a small mirror as a platform. C-ploymodal nociceptors (C-PMNs) were characterized by their response to form moderate to strong pressure as well as to heat stimuli. Conduction velocities of these units were 0.51-1.03 $m-^{1}s$. Receptive fields were carefully mapped and mechanical thresholds were determined in millinewtons (mN) using calibrated von Frey bristles. Controlled mechanical forces (10-100 mN) were applied to the receptive fields of the units for 4.5/ seconds at 1-2 minutes intervals using a motorized stimulator (Fitzgerald and Lynn 1977) with 0.8/ mm probe diameter. Mechanical response of these units was assessed by the number of impulses evoked during the period of application of the above repeated forces. Eight to fifteen stimuli were applied before hypotension (normal blood pressure), 9-21 stimuli during hypotension (severe reduction in blood pressure), and 10-26 stimuli applied after hypotension (restoring the normal blood pressure). The number of stimuli was selected in order to establish a reliable level of discharge to

the repeated stimuli. The discharge evoked by these units was monitored on a digital storage oscilloscope (Tektronix 2230) and plotted by a chart recorder (Dash IV, Astro, Med, Inc.) alongside with the blood pressure signal.

Acute hypotension

The normal systolic blood pressure of these rats was 120-140 mm Hg (131.0 mm Hg ± 2.5, n = 10).Severe acute reduction in the blood pressure (hypotension) was induced by withdrawing about 3.5-4.0 ml of blood through the cannula in the common carotid artery using a syringe containing 1 ml of heparinized saline (150 units^{-1} ml). This procedure caused immediate decrease in the systolic pressure to only 40-60 mm Hg (51.2 mm Hg ± 2.3, n = 10). Ten to sixty minutes later, the whole blood was returned to the animal through the same cannula raising the blood pressure to its normal level of 140-160 mm Hg (142.8 mm Hg ± 4.6, n = 10). Data were expressed as means ± standard error of the mean (S.E.M.)

Results

Mechanical thresholds of the rat C-PMNs were compared before, during and after acute hypotension (Fig.1). The mean von Frey pressure thresholds of these units before hypotension (9.7 mN ± 2.2 mN, n = 10) was not significantly different from the mean of pressure during and after acute hypotension (12.0 mN ± 2.3 mN, n = 10), $P > 0.1$, t-test. On the other hand, the mechanical response of the C-PMNs to repeated controlled mechanical forces (10-100 mN) was reduced in 9 units (out of 10 units) during and after acute hypotension (Fig. 2). A typical response of one C-PMN unit to repeated 55 mN force applied before, during and after hypotension is shown in Fig. 3. The discharge of this unit to the above force was not fully recovered to its normal level even after 15 minutes of restoring the normal blood pressure (Fig. 4). The discharge of 9 C-PMN units to the repeated controlled forces was reduced during and after acute hypotension compared to their normal response of pre-hypotension level. The mean number of spikes evoked by these units to mechancial stimulation before hypotension (15.16 spikes ± 1.71, n = 9) is significantly higher than that during (9.97 spikes ± 1.32, n = 9), or after hypotension (10.44 spikes ± 1.47, n = 9), $P < 0.025$, Mann-Whitney test. The overall reduction in the mechanical response (Fig. 5) was 65.6% ± 3.3% (n = 9) during hypotension, and to 68.8% ± 3.1% (n = 9) after hypotension, $P < 0.025$, t-test. However, the mechanical response of only one C-PMN unit, out of the 10 units, was not affected by the reduction in blood pressure.

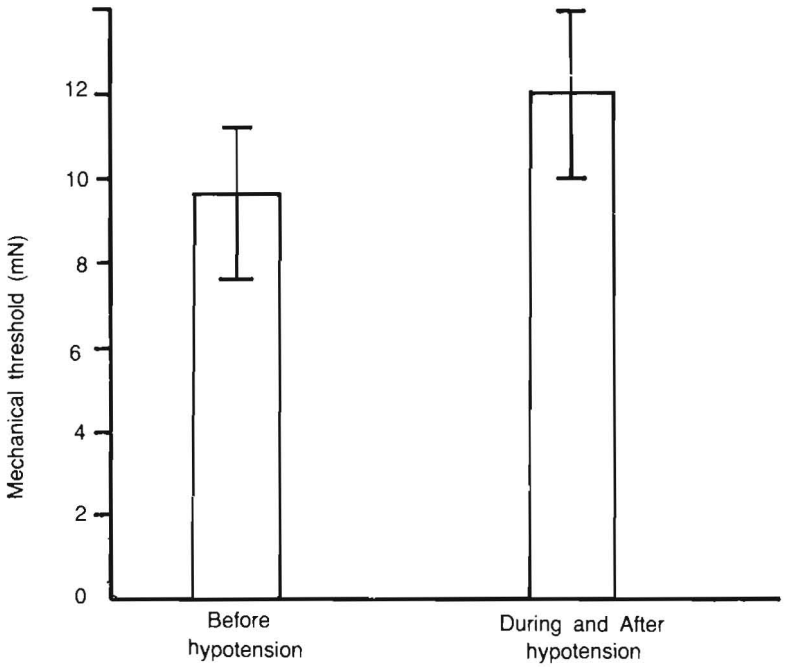

Fig. 1. Histogram of mean mechanical thresholds (mN) of the rat C-polymodal nociceptors (C-PMNs) before, during and after acute hypotension. Mechanical thresholds were determined using calibrated von Frey bristles. Error bars represent ±Standard Error of Mean (S.E.M.).

Discussion

The present study has examined the effect of acute hypotension on the mechanical response of the rat C-PMNs. During Severe reduction in blood pressure, the discharge of these units to repeated controlled mechanical forces was reduced compared to their response of pre-hypotension level, However, the heat response of these units in the rabbit were not affected by the local changes in the blood flow (Lynn 1979). Similarly, under the conditions of opiate drug treatment in the cat, the pattern of discharge of these units was consistent with repeated heating (Senami *et al.* 1986) and with strong mechanical stimulation in the rat (Shakhanbeh and Lynn, 1992). The discrepancy in the findings between the mechanical response of the rat C-PMNs in the present study and the heat response of similar units described in the rabbit (Lynn 1979) might indicate that, although these two modalities of stimuli are mediated by the same nociceptive nerve fibers, yet different membrane mechanisms are probably involved. This is in agreement with the findings on ionic mechanisms of excitation of primary afferent units (Szolcsanyi 1987, Sato *et al.* 1989, Lang *et al.* 1990 and Belmonte *et al.* 1991). Alternatively, the excitability of the C-PMNs by mechanical stimulation might require more energy than that needed for heat stimulation.

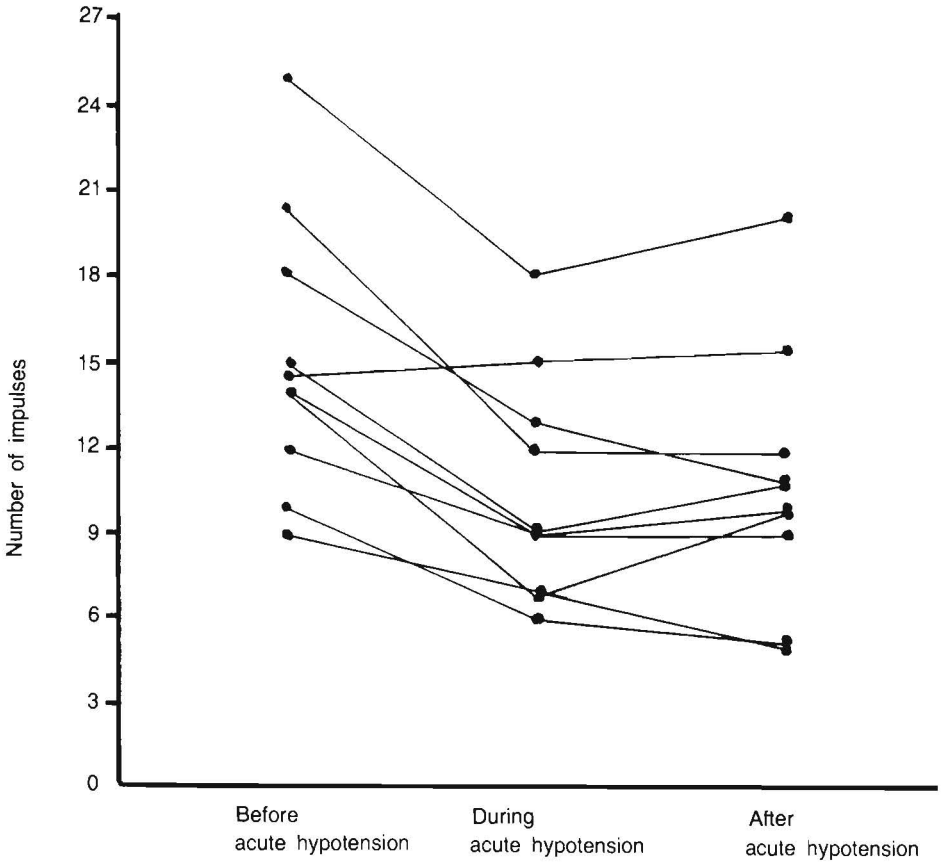

Fig. 2. Effect of acute hypotension on the discharge of C-PMNs in the rat skin to repeated controlled forces (10-100 mN). Each point represents the mean of 8-15 responses before hypotension, 9-21 responses during hypotension and 10-26 responses after hypotension.

The decrease in the discharge of the rat C-PMNs to repeated mechanical forces caused by the hypotension might be due to the resulting anoxia and to the changes in the local environment near the endings of these nerve fibers as a result of accumulation of metabolites. Since the reduction in oxygen supply is known to be associated with hyperpolarization of the membrane potential of nerve cells (Hansen *et al.* 1982, Fujiwara *et al.* 1987, Krnjevic and Leblond 1989, Leblond and krnjevic 1989, and Cowan and Martin 1992). The present results demonstrated the inability of C-PMNs to recover normal mechanical responses even after one hour of restoring normal blood pressure. This might be due to an irreversible short term inhibition evoked on the mechanical excitability of these units by severe reduction in blood pressure. Moreover, only the nociceptive mechanical response has been affected but not the von Frey pressure thresholds. This might suggest that the whole process of mechanical excitability could be related to the amount of energy of stimulation acting through different mechanical transduction mechanisms.This is supported by the recent findings of White and Levine (1993) which showed that only sustained subthreshold but not high force of stimulation induced sensitization of the rat C-PMNs. The present results might indicate that the response of the rat C-PMNs to strong repeated mechanical forces is markedly affected by the changes in their peripheral vascular conditions resulting from the reduction in oxygen supply together with the consequent accumulation of metabolites.

Acknowledgements

This work was supported by the British council in Jordan and Mu'tah University.

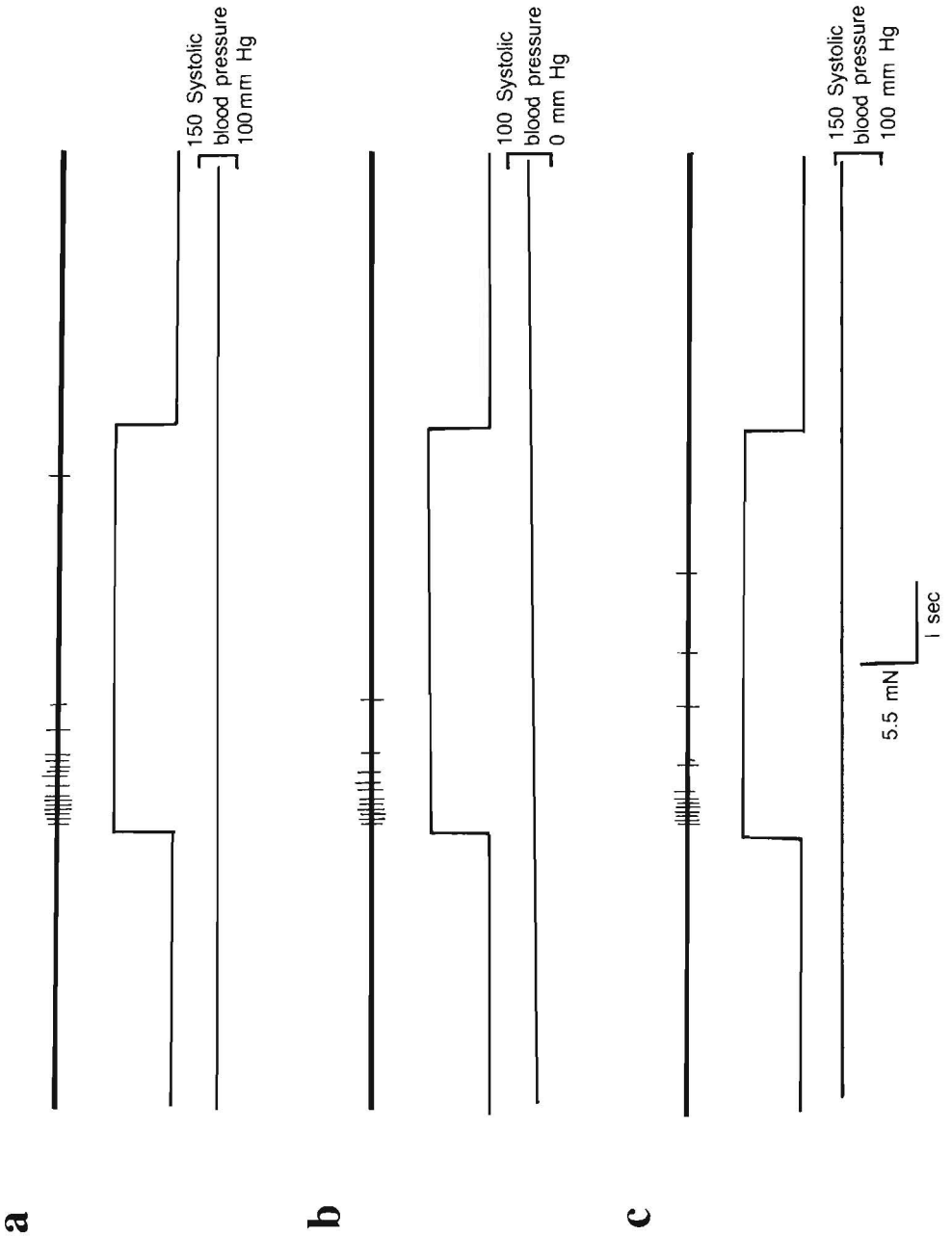

Fig 3. Typical response of one C-PMN unit to repeated 55 mN force applied before (c), during (b) and after (a) acute hypotension. Upper trace in each figure represents the discharge evoked by the unit, middle trace represents the force profile and lower trace represents the systolic blood pressure (mm Hg).

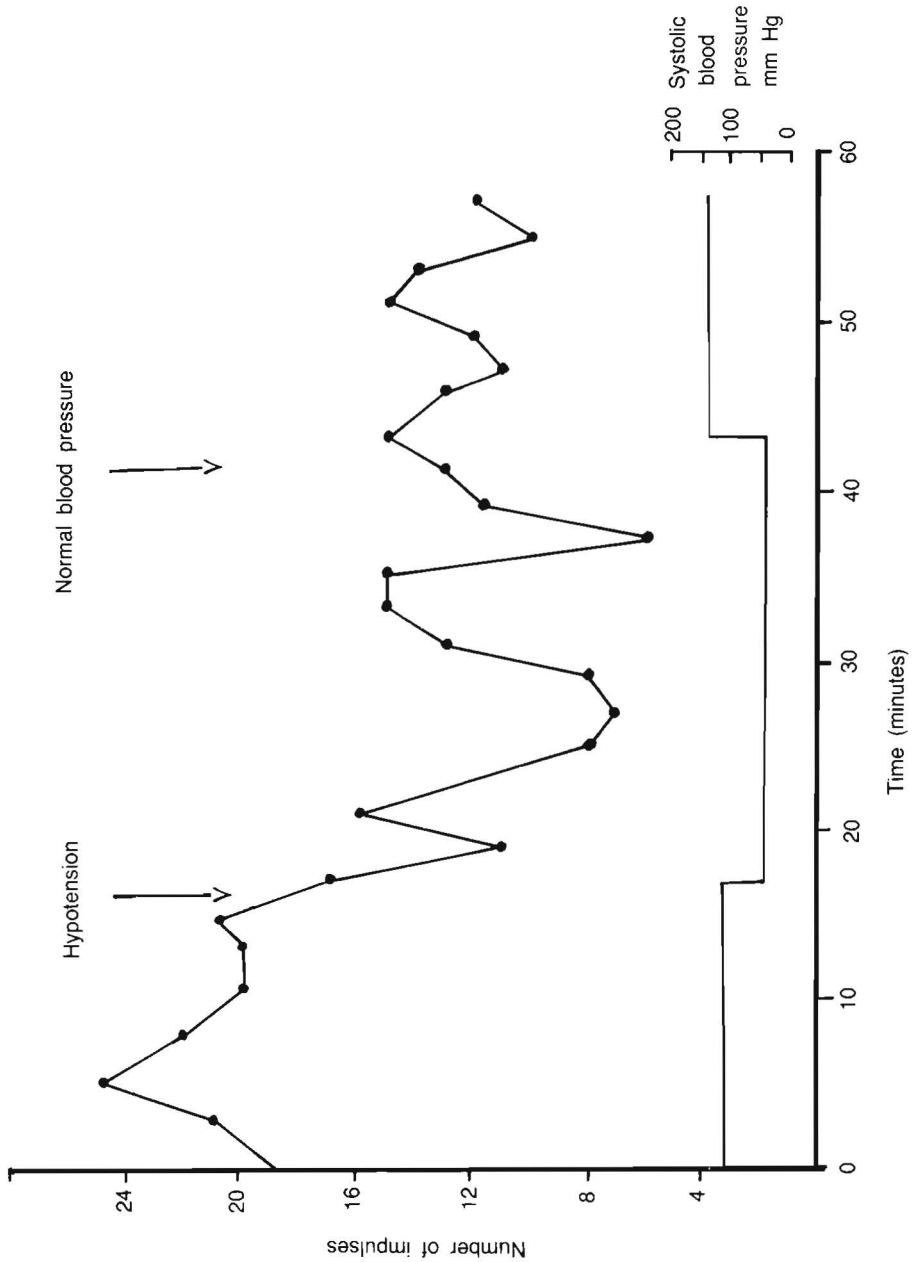

Fig 4. Response of one C-PMN unit to repeated 55 mN force applied before, during and after acute hypotension. Upper trace represents the number of impulses evoked by the unit. Lower trace shows the systolic blood pressure (mm Hg).

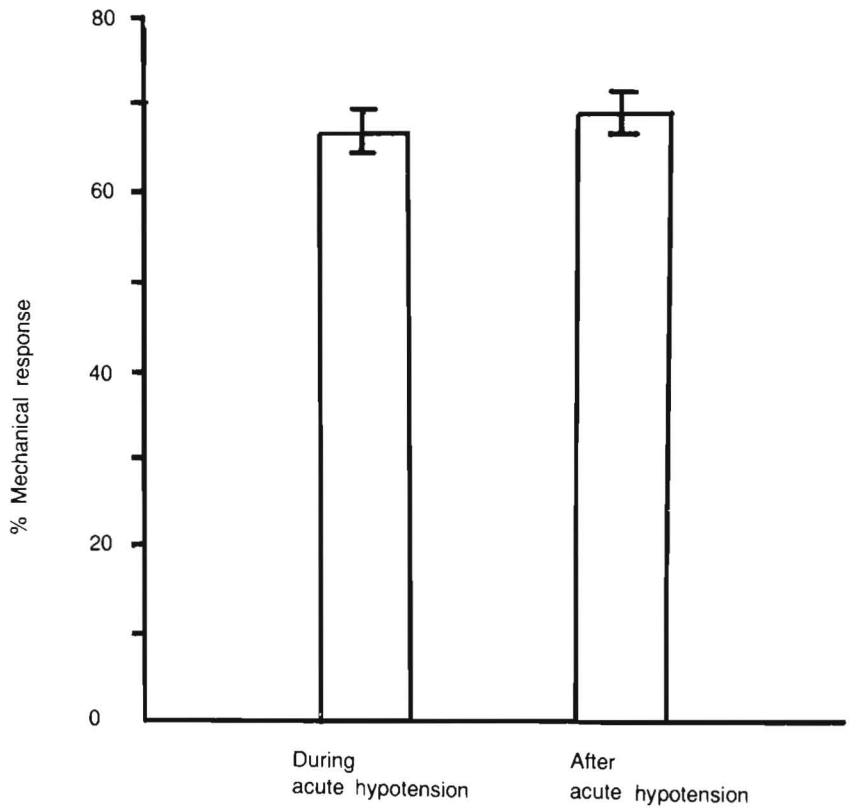

Fig 5. Histogram of mean mechanical response of 9 C-PMNs (expressed as percentage of their response of pre-hypotension level) to repeated controlled forces (10-100 mN) applied during and after acute hypotension. Error bars represent ±S.E.M.

References

Adriaensen, H., Gybels, J., Handwerker, H.O. and **Van Hees, J.** (1984) Suppression of C-fiber discharges upon repeated heat stimulation may explain characteristics of concomitant pain sensations. *Brain Res.* **302:** 203-211.

Beitel, R.E. and **Dubner, R.** (1976) Fatigue and adaptations in unmyelinated C polymodal nociceptors to mechanical and thermal stimuli applied to monkey's face. *Brain Res.* **112:** 402-406.

Belmonte, C., Gallar, J., Pozo, M.A. and **Rebollo, I.** (1991) Excitation by irritant chemical substances of sensory afferent units in the cat's cornea. *J. Physiol.* **437:** 709-725.

Campbell, J. N., Khan. A. A. Meyer, R. A. and **Raja, S.** (1988) Responses to heat of C-fiber nocicepotrs in monkey are altered by injury in the receptive field but not by adjacent injury. *Pain* **32:** 327-332.

Campbell, J.N. and **Meyer, R.A.** (1983) Sensitization of unmyelinated nociceptive afferents in monkey varies with skin type. *J. Neurophysiol.* **49:** 98-110.

Carter, D.A. and **Lisney, S.J.W.** (1987) The numbers of unmyelinated and myelinated axons in normal and regenerated rat saphenous nerves. *J. Neurol. Sci.* **80:** 163-171.

Chad, D., Bradly, W.G., Rasool, C., Good, P., Reichlin, S. and **Zivin, J.** (1983) Sympathetic postganglionic unmyelinated axons in the rat peripheral nervous system. *Neurology* **33:** 841-847.

Cowan, I.A. and **Martin, R.L.** (1992) Ionic bases of membrane potential changes induced by anoxia in rat dorsal vagal motoneurones. *J. Physiol.* **455:** 89-109.

Croze, S., Duclaux, R. and **Kneshalo, D.R.** (1976) The thermal sensitivity of the polymodal nociceptors in the monkey. *J. Physiol.* **263:** 539-562.

Fitzgerald, M. (1979) The spread of sensitization of polymodal nociceptors in the rabbit from nearby injury and by antidromic nerve stimulation. *J. Physiol.* **297:** 207-216.

Fitzgerald, M. and **Lynn, B.** (1977) The sensitization of high threshold mechanoreceptors with myelinated axons by repeated heating. *J. Physiol.* **365:** 549-563.

Fleischer, E., Handwerker, H.O. and **Joukhadar, S.** (1983) Unmyelinated nociceptive units in two skin areas of the rat. *Brain Res.* **267:** 81-92.

Fujiwara, N., Higashi, H., Shimoji. K. and **Yoshimura. M.** (1987) Effects of hypoxia on rat hippocampal neurons *in vitro*. *J. Physiol.* **384:** 13-151.

Handwerker, H.O. and **Neher, K.D.** (1976) Characteristics of C-fiber receptors in the cat's foot responding to stepwise increase of skin temperature to noxious levels. *Pufluger's Arch.* **365:** 221-229.

Hansen, A.J., Hounsgaard, J. and **Jainsen, H.** (1982) Anoxia increases potassium conductance in hippocampal nerve cells. *Acta Physiol. Scand.* **115:** 301-310.

He, X, Schmidt, R. F. and **Schmittner, H.** (1988) Effects of capsaicin on articular afferents of the cat's knee joint. *Agents and Actions* **25:** 222-224.

Kenins, P. (1982) Responses of single nerve fibers to capsaicin applied to the skin. *Neurosci. Lett.* **29:** 83-88.

Kirchhoff, C., Kessler, W., Jung, S., Reeh, P.W. and **Handwerker, H. O.** (1988) Inflammatory sensitization of rat polymodal nociceptors to bradykinin. *Pfluger's Arch.* **412** (supp. 1): R76.

Kocher, L., Anton, F., Reeh, P.W. and **Handwerker, H.O.** (1987) The effect of carrageenan-induced inflammation on the sensitivity of unmyelinated skin nociceptors in the rat. *Pain* **29:** 363-373.

Krnjevic, K. and **Leblond. J.** (1989) Changes in membrane currents of hippocampal neurons evoked by brief anoxia. *J. Neurophysiol.* **62**: 15-30.

Lamotte, R.H., Thalhammer, J.G. and **Robinson. C.J.** (1983) Peripheral neural correlates of magnitude of cutaneous pain and hyperalgesia: a comparison of neural events in monkey with sensory judgments in human. *J. Neurophysiol.* **50:** 1-26.

Lang, E., Novak, A., Reeh, P.W. and **Handwerker, H.O.** (1990) Chemosensitivity of fine afferents from rat skin *in vitro*. *J. Neurophysiol.* **63:** 887-901.

Leblond, J. and **Krnjevic, K.** (1989) Hypoxic changes in hippocampal neurons. *J. Neurophysiol.* **62:** 1-14.

Lynn, B. (1979) The heat sensitization of polymodal nociceptors in the rabbit and its independence of the local blood flow. *J. Physiol.* **287:** 493-507.

Lynn, B. (1984) Effect of neonatal treatment with capsaicin on the numbers and properties of cutaneous afferent units from the hairy skin of the rat. *Brain Res.* **322:** 255-260.

Lynn, B. and **Carpenter, S.E.** (1982) Primary afferent units from the hairy skin of the rat hind limb. *Brain Res.* **238:** 29-43.

Pozo, M.A., Gallego, R., Gallar, J. and **Belmonte, C.** (1992) Blockade by calcium antagonists, of chemical excitation and sensitization of polymodal nociceptors in the cat's cornea. *J. physiol.* **450:** 179-189.

Russell, N.J.W., Schaible, H. G. and **Schmidt, R.F.** (1987) Opiates inhibit the discharges of fine afferent units from inflamed knee joint of the cat. *Neurosci. Lett.* **76:** 107-112.

Sato, J., Mizumura, K. and **Kumazawa, T.** (1989) Effect of ionic calcium on the response of canine testicular polymodal receptors to algesic substances. *J. Neurophysiol.* **62:**119-125.

Senami, M., Aoki, M., Kitahata, L.M., Collins, J.G., Kumeta, Y. and **Murata, K.** (1986) Lack of opiate effects on cat C-polymodal nociceptive fibers. *Pain* **27:** 81-90.

Shakhanbeh, J. and **Lynn, B.** (1992) Morphine inhibits antidromic vasodilatation without affecting the excitability of C-polymodal nociceptors in the skin of the rat. *Brain Res.* **607:** 314-318.

Shea, V.K. and **Perl, E.R.** (1985) Sensory receptors with unmyelinated C-fibers innervating the skin of the rabbit's ear. *J. Neurophysiol.* **54:**491-501.

Szolcsanyi, J. (1987) Selective responsiveness of polymodal nociceptors of the rabbit ear to capsaicin, bradykinin and ultra-violet irradiation. *J. Physiol.* **388:** 9-23.

Szolcsanyi, J., Anton, F., Reeh, P.W. and **Handwerker, H.O.** (1988) Selective excitation by capsaicin of mechano-heat sensitive nociceptors in rat skin. *Brain. Res.* **446:** 262-268.

Torebjork, H.E. (1974) Afferent C units responding to mechanical, thermal and chemical stimuli in human non-glabrous skin. *Acta Physiol. Scand.* **92**: 374-390.

White, D.M. and **Levine. J.D.**(1993) Separate mechanical transduction mechanisms for the immediate and delayed responses of rat C- fiber nociceptors. *7th World Congress on Pain, Abstr.* **376:** 142-143.

(Received 09/03/1993;
in revised form 19/10/1993)

تأثير إنخفاض ضغط الدم الحاد على الإستجابة الآلية لمستقبلات الألم متعددة الأنماط في جلد الجرذ

جمعة الشخانبة

قسم العلوم الحياتية ـ كلية العلوم ـ جامعة مؤتة
ص.ب: ٧ ـ الكرك ـ المملكة الاردنية الهاشمية

لقد تمّت دراسة تأثير الانخفاض الحاد المفاجىء في ضغط الدم على الإستجابة الآلية لمستقبلات الألم متعددة الأنماط الموجودة في جلد الجرذ. لقد تم تخدير الجرذان (التي تراوحت أوزانها من ٢٠٠ إلى ٥٥٠ غم) بالصوديوم بينتوباربيتال (٥٠ ملغم/كغم) ثم قياس وتثبيت درجة حرارة جسم الحيوان على درجة ٣٧°م، وكذلك تم قياس ومراقبة ضغط الدم عن طريق الشريان الدموي السباتي. وبعد ذلك تم كشف العصب الأسفيني (الفخذي) وتشريحه والتعرف على ١٠ وحدات عصبية حسيّة من نوع مستقبلات الألم متعددة الأنماط الجلدية.

وقد تم تحديد مقدار عتبتها الآلية (بإستعمال شعيرات فون فراي المدرجة) قبل وبعد فترة إنخفاض الضغط الدموي. كما تم أيضاً معرفة مقدار إستجابة هذه المستقبلات (عن طريق عدد السيالات العصبية التي تطلقها) عند تعريضها لمثيرات آلية ثابتة الشدة (تراوحت قيمتها من ١٠ إلى ١٠٠ ميلينيوتن) ومتكررة على فترات زمنية من دقيقة واحدة إلى دقيقتين، حيث كان عدد مرات تأثير هذه القوى على المساحة الحسيّة الجلديّة لهذه المستقبلات هو ٨ ـ ١٥ مرة قبل إنخفاض الضغط الدموي، و٩ ـ ٢١ مرة خلال فترة إنخفاض الضغط الدموي، و١٠ ـ ٢٦ مرة بعد مرحلة أنخفاض الضغط الدموي.

وقد كان زمن تأثير كل من هذه القوى في كل مرة هو ٥ , ٤ ثانية. هذا وقد تم احداث الإنخفاض الحاد المفاجىء في ضغط الدم عن طريق سحب ٥ , ٣ ـ ٤ ميلليلتر من الدم بواسطة محقن طبي (يحتوي على ١ ميلليلتر من المحلول الملحي الفسيولوجي الهيباريني) موصول بواسطة أنبوبة مطاطية صغيرة مع الشريان الدموي السباتي. وقد أدت هذه العملية إلى إنخفاض حاد ومفاجىء في ضغط الدم الانقباضي وصل ٤٠ ـ ٦٠ ميليمتر زئبق مقارنة بالضغط الدموي العادي وهو ١٢٠ ـ ١٤٠ ميليمتر زئبق قبل عملية سحب الدم. وقد تراوحت فترة الإنخفاض في ضغط الدم ١٠ ـ ٦٠ دقيقة تم خلالها تعرض هذه المستقبلات للمثيرات الآلية المتكررة المذكورة، وذلك لمعرفة مقدار استجابتها الآلية تحت ظروف إنخفاض الضغط الدموي. وبعد هذه الفترة تم استرجاع جميع كمية الدم المسحوبة إلى الجرذ عن طريق نفس الشريان المذكور، مما أدى إلى استعادة ضغط الدم إلى مستواه الطبيعي السابق.

تبين نتائج هذه الدراسة أن العتبة الآلية لمستقبلات متعددة الألم الموجودة في جلد الجرذ لم تتأثر بعملية الإنخفاض الحاد المفاجىء في الضغط الدموي. حيث لم يكن هنالك فارق إحصائي بين متوسط العتبة الآلية لهذه المستقبلات، قبل إنخفاض ضغط الدم ٧ , ٩ ميلينيوتن ± ٢ , ٢ (ن = ١٠) ومتوسط عتبتها الآلية خلال فترة أنخفاض الضغط الدموي أو ما بعدها ٠ , ١٢ ميلينيوتن ± ٣ , ٢ (ن = ١٠). أما بالنسبة إلى استجابة هذه المستقبلات للمثيرات الآلية المتكررة السابقة الذكر فلقد أدى إنخفاض الضغط الدموي الحاد إلى نقصانٍ ملحوظٍ في مقدارِ الأستجابة الآلية لتسعة وحدات (من بين العشرة وحدات المدروسة)، مقارنةً مع مقدار إستجابتها الآلية لنفس المثيرات قبل عملية إنخفاض الضغط الدموي، حيثُ نقص المتوسط المئوي للإستجابة الآلية لهذه المستقبلات إلى ٦ , ٦٥٪ ± ٣ , ٣ (ن = ٩) خلال فترة الإنخفاض في الضغط الدموي، وإلى ٨ , ٦٨٪ ± ١ , ٣ (ن = ٩) بعد فترة الإنخفاض في الضغط الدموي (أي بعد إستعادة ضغط الدم إلى مستواه الطبيعي).

لقد دلّت نتائج هذه الدراسة على أن الإستجابة الآلية لمستقبلات الألم متعددة الأنماط الموجودة في جلد الجرذ تتأثر سلبياً بظروف إنخفاض الضغط الدموي. وقد يرجع ذلك إلى نقصان في كمية الأوكسجين، أو لتراكم مخلفات مواد الأيض الخلوية المتكونة في المنطقة المحيطة بنهايات الألياف العصبية لهذه المستقبلات، نتيجةً للإنخفاض في الضغط الدموي.