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Long lasting activity of nociceptive muscular afferents facilitates bilateral flexion reflex pattern in the feline spinal cord

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ABSTRACT

Chronic muscular limb pain requires the adoption of motor patterns distinct from the classic ipsilateral flexion, crossed extension and corresponding reciprocal inhibitions to acute exteroceptive stimulation. Using selective chemical activation of group III/IV afferents in gastrocnemius-soleus (GS) muscles we investigated bilaterally their reflex responses conditioned by (a) acute 'myositis' induced by intramuscular carrageenan; and (b) sub-acute 'myositis' induced by infusion of complete Freund's adjuvant (CFA). Reflex transmission was detected by monosynaptic testing and c-fos staining used to identify increased neuronal activity. In all control experiments with chemical stimulation of group III/IV afferents, ipsilateral responses conformed to the flexor reflex pattern. However, the expected contralateral facilitation of GS motoneurones occurred in fewer than 50% trials while only 9% of trials induced contralateral inhibition of flexor posterior-biceps-semitendinosus (PBSt) motoneurones. During carrageenan acute myositis contralateral PBSt was transiently facilitated by selective activation of group III/IV afferents. During CFA-induced myositis, contralateral only inhibition of GS motoneurones occurred instead of any facilitation, while bidirectionally a crossed facilitation of PBST dominated. These reflex changes were mirrored in an enhanced number of neurones with enhanced c-fos expression. Muscle pain, particularly if chronically persistent, requires another behavioural response pattern than acute exteroceptive pain.

1. Introduction

Ipsilateral flexion and extensor inhibition coupled with crossed extension is the characteristic reflex response to cutaneous nociceptive stimulation. However, it turned out that these and other afferents may also exert reciprocal reflex effects by using alternative interneuronal pathways for reflex transmission under different experimental conditions (Schomburg, 1990; Sandrini et al., 2005). Similarly, the crossed extension reflex is not a unique response, instead, crossed inhibition of extensors might occur under a variety of conditions (Duysens et al., 1980; Frigon and Rossignol, 2008). In such studies exteroreceptor stimulation of non- and nociceptive afferent pathways has mainly been cutaneously activated by electrical nerve or mechanical stimulation (Harris et al., 2014). In the case of muscle selective chemical activation of group III and IV muscle afferents by intra-arterial injection of KCl into the gastrocnemius-soleus (GS) muscle (Mense, 1977) evoked a clearly predominant ipsilateral flexion reflex pattern with flexor excitation and extensor inhibition (Kniffki et al., 1981a; Schomburg et al., 2012). Still missing, however, is a systematic analysis of the contralateral reflex responses particularly with respect to their consistency. The aim of this study was to investigate both the ipsilateral and bilateral reflex effects of chemically activated muscle afferents and the conditioning influence on them by muscle inflammation. The influence of carrageenan induced acute muscle inflammation (Diehl et al., 1988) was compared with the influence of the longer lasting sub-acute ("chronic") muscle inflammation induced by infiltration of complete Freund's adjuvant (CFA) (Chacur et al., 2009).

Abbreviations: CFA, complete Freund's adjuvant; FRA, flexor reflex afferents; GS, gastrocnemius-soleus muscle/nerve; PBSt, posterior biceps semitendinosus nerve.

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The number and distribution of c-fos immunoreactive cells have proved useful in the analysis of long-term alterations in the nervous system (Gao and Ji, 2009; Harris et al., 2014), e.g., an enhanced expression of c-fos has been demonstrated in spinal neurones following prolonged nociceptive and non-nociceptive stimulation (Herdegen et al., 1994; Pilyavskii et al., 2001; Schomburg et al., 2007). So to gain further insight as to possible structural mechanisms involved in the current modifications of reflex transmission the electrophysiologically collected data have been correlated with the early gene c-fos proto-oncogene expression at the transition from acute to chronic muscle pain as described in previous studies (Schomburg et al., 2007).

2. Methods

2.1. Ethics statement

The experiments were performed according to the ethical guidelines of the national animal protection law and were authorised by the ethical committee of the State of Lower Saxony (review board institution: Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, Dezernat 33, Oldenburg, Germany) and the ethics committee of the University of Göttingen.

2.2. General procedure

The experiments were carried out on adult cats (2.4–6.7 kg, mean 3.3 kg; age 2–3.5 years). Electrophysiological investigations were performed in four groups: (a) Controls: (a1) cats with only unilateral preparation and stimulation without any inflammatory conditioning ($n = 4$, data in Fig. 7A from "sham-operated group" from Schomburg et al., 2007); (a2) cats with bilateral preparation and stimulation without ($n = 4$) or before any inflammatory conditioning, i.e. before carrageenan application ($n = 6$); (b) experiments with unilateral infiltration of the left GS muscle with 5 ml carrageenan (1–2% in Ringer's solution) and bilateral stimulation and recording ($n = 6$, 3 of them from Schomburg et al., 2012) or only unilateral stimulation and recording ($n = 4$, in Fig. 7B, "carrageenan-injected group" from Schomburg et al., 2007) in each experiment with control tests before infiltration. The later 4 animals and the 4 control animals of "a1" had provided data before for electrophysiology and c-fos (Schomburg et al., 2007); (c) experiments with infiltration of the left GS muscle with 1 ml of complete Freund's adjuvant (CFA, modified with *Mycobacterium butyricum* [Calbiochem, USA] dissolved in 1 ml of Ringer's solution; three injections of 0.2 ml into each head of the muscle under full anaesthesia (inhalation anaesthesia as described below)) 9–12 days before the terminal electrophysiological experiments ($n = 4$). In all groups (a, b and c) some cats were perfused after the electrophysiological experiments for determination of c-fos activity (Fig. 7): (a1) Controls ($n = 4$); (b) carrageenan infiltration of left GS ($n = 4$); (c) CFA infiltration of left GS ($n = 3$). After infiltration of the GS muscle with CFA the cats came back to their familiar surroundings remaining under permanent veterinary control with at least one inspection per day. The cats were living together with untreated cats in a group of 4–6 animals in a room of about12 m^2. Like the untreated cats they were mainly lying about on the provided resting places. A difference in explorative spontaneous locomotor activity between naive and myositis animals observed in rats (Chacur et al., 2009) was therefore not obvious in the cat. But if moving for feeding, drinking and apart from that they were moving around without any particular signs of impairment. This coincides with the observation that locomotion on the rotating rod was not impaired by muscle inflammation in rats (Chacur et al., 2009). They did not show any weight loss and could be handled in the same way as untreated animals, except that like in rats (Chacur et al., 2009) touch or pressure of the affected muscle was not tolerated and was obviously painful. All together, it can be assumed that the animals were quite adapted to the developing continuous nociceptive input from the CFA infiltrated muscle without feeling distinct permanent pain.

For the terminal experiments, cats were first anaesthetised by inhalation of an ether–halothane-nitrous oxide mixture (O_2/N_2O, 1:2; halothane initially 2.5%, then progressively replaced by ether for full anaesthesia). As previously described for a series of experiments (Kniffki et al., 1981b), anaemic decapitation was performed by ligation of the common carotids and their branches together with permanent clamping of the vertebral arteries at the level of C2 vertebra. For further technical details see Schomburg et al. (2007) and Fig. 1. At the end of the experiment the cats were either sacrificed by i.v. injection of 5 ml of a 3 M KCl solution, or perfused with a formalin solution for determination of *c-fos* immunoreactivity (see below).

2.3. Preparation and electrical stimulation

The ventral roots L5-S1 were sectioned on both sides and the combined L7/S1 ventral roots on either side mounted (Fig. 1 (6)/(6a)) for bilateral recording of the monosynaptic reflexes of posterior biceps semitendinosus (PBSt) and GS nerves. Except for the experiments with only unilateral preparation and stimulation (group a1 and partly group b; see Section 2.2), both hind-limbs were completely denervated below the hip except for the nerves to the medial and lateral GS. The proximal end of the transected muscle nerve to the flexor PBSt (Fig. 1 (4)/(4a)) and the intact nerve to the extensor GS (Fig. 1 (5)/(5a)), on both sides were mounted on bipolar platinum wire electrodes and electrically stimulated with rectangular pulses of 0.1 ms duration and a repetition rate of around 0.5 Hz. The stimulus strength is indicated in multiples of the threshold strength (T) for stimulating the lowest threshold fibres of the corresponding nerve. The incoming volley was recorded at the L7 dorsal root entry (Fig. 1 (7)). Chemical stimulation of group III and IV muscle receptors was performed according to the technique described by Kniffki et al. (1981a).

2.4. Testing procedure

Central transmission in group III and IV pathways was examined using monosynaptic reflex testing. The PBSt and GS nerves on either side were stimulated at intervals of 1.8 s and at 5 T, i.e. well above group I maxima; and the corresponding monosynaptic reflexes were each recorded from the combined ventral roots L7/S1 with or without conditioning by chemical activation of group III and IV muscle afferents with KCl (Fig. 1). Amplitudes of monosynaptic reflexes were measured peak to peak. After 1–2 h of control recordings the GS muscle was infiltrated with carrageenan. For determining the percentage change in amplitudes of these conditioned reflexes the last eight reflex responses before KCl injection were averaged and that value was defined as 100%.

2.5. Perfusion and c-fos immunohistochemistry

The final perfusion and determination of c-fos immunohistochemistry were performed according to the technique described by Schomburg et al. (2007). In four animals with carrageenan-induced myositis the perfusions were started about 11 h after the onset of the operative procedure which included 2 h of control recording and 4–5 h after the carrageenan infiltration of GS. Four control animals without carrageenan infiltration were perfused at corresponding times after undergoing identical experimental procedures. Three animals were perfused 9–12 days after prior