Evidence that the contraction-induced rapid hyperemia in rabbit masseter muscle is based on a mechano-sensitive mechanism, not shared by cutaneous vascular beds.

Evidence that the contraction-induced rapid hyperemia in rabbit masseter muscle is based on a mechano-sensitive mechanism, not shared by cutaneous vascular beds

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Abstract

Several mechanisms have been hypothesized to contribute to the rapid hyperemia at the onset of exercise. Aim of the present study was to investigate the role played by the mechano-sensitivity of the vascular network.

In 12 anesthetized rabbits blood flow was recorded from the exclusively muscular masseteric artery in response to brief spontaneous contractions (BSC) of the masseter muscle, artery occlusion (AO), muscle compression (MC) and muscle stretch (MS). Activation of masseter muscle was monitored by electromyography (EMG). Responses to AO were also recorded from the mostly-cutaneous facial and the central ear arteries. Five animals were also tested in the awake condition.

The hyperemic response to BSC (peak amplitude of 394 ± 82%; time-to-peak of 1.8 ± 0.8 s) developed with a latency of 300-400 ms from the beginning of the EMG burst and 200-300 ms from the contraction-induced transient flow reduction. This response was neither different from the response to AO (peak amplitude = 426 ± 158 %), MC and MS ($p=0.23$), nor from the BSC response in the awake condition. As compared to the masseteric artery, the response to AO was markedly smaller both in the facial (83 ± 18 %,) and in the central ear artery (68 ± 20 %) ($p<0.01$).

In conclusion, the rapid contraction-induced hyperemia can be replicated by a variety of stimuli affecting transmural pressure in muscle blood vessels and is thus compatible with the Bayliss effect. This prominent mechano-sensitivity appears to be a characteristic of muscle and not cutaneous vascular beds.

K**eywords:** muscle stretch, myogenic response, rapid dilatation, reactive hyperemia, muscle compression

Introduction

Skeletal muscle blood flow has long been known to exhibit a rapid increase at the onset of exercise (1, 9, 12, 22, 27). The mechanisms behind this phenomenon are still matter of debate in spite of the many new insights provided by different research groups in the recent years. In particular it has been shown that the contraction-induced compression of venous compartments (muscle pump) cannot fully account for the blood flow increase and that the additional occurrence of a rapid active dilatation is required (18, 33, 45, 46). Other studies have shown that this rapid dilatation is mediated neither by changes in sympathetic neural drive (7, 41), nor by ACh spillover from the motor endplate (6, 33), nor by endothelium-released NO (6), although this last assertion is debated (see Discussion). As early as 1974, Mohrman and Sparks (31) hypothesized that the rapid dilatation could result from the myogenic response of muscle blood vessels to decreased transmural pressure, which in turn results from the increase in intramuscular (extravascular) pressure produced by the contraction. They showed, in fact, that the rapid hyperemia provoked by a 1-s tetanic stimulation of the isolated calf muscle of the dog could be reproduced, although with lower magnitude, by a brief external compression of the muscle performed through an inflatable cuff (31). The concept of a mechanically-induced dilatation has recently found renewed support, based on *in-vitro* (10) as well as *in-vivo* studies performed on several animal models (19, 33) including humans (6, 11, 24). In particular it has been shown that the increase in extravascular pressure could induce the rapid dilatation of an isolated muscle artery (10) and that the rapid blood flow increase evoked by a brief hand grip was closely reproduced by a brief external compression of the forearm (6, 24). These data led some authors to re-consider the myogenic response as one of the possible mechanisms behind the exercise-induced rapid dilatation (10, 11, 14). For the sake of clarity, it should be emphasized that we here refer to the *rapid* myogenic response that, as first reported by Bayliss, can be evoked *in vivo* by a short lasting arterial occlusion in the form of a rapid (reactive) hyperemia (3, 5, 38). At difference with the "classical" (slow) myogenic response, which develops over a period in the order of 1 minute upon sustained changes in transmural pressure and which has been extensively studied

and characterized in a variety of experimental conditions, the *rapid* myogenic response has received much less consideration (15, 21) and is still poorly understood.

However, a number of intrinsic limitations in the experimental models make the involvement of the mechano-sensitive mechanisms in the contraction-induced hyperemia difficult to quantify. First of all, external compression of the forearm is not an unequivocal mean to induce a myogenic response since the compression-induced depletion of venous compartments may contribute to the hyperemia according to the muscle pump mechanism (26, 45). Surprisingly, the contraction induced hyperemia has never been directly compared to the response to artery occlusion, which would overcome this limitation. Secondly, since blood flow (or blood velocity) is commonly recorded from large arteries supplying muscolo-cutaneous vascular compartments, it remains to be assessed to what extent response to compression of cutaneous tissues may contribute to the observed effects. A third issue concerns the accuracy in detecting the latency of the rapid dilatation with respect to muscle contraction, which is crucial to discriminate between the faster mechanical *versus* the slower metabolic mechanism. Based on different experimental models, such latency is currently reported to be "< 1 s" (24, 33, 41, 47), which is considered too short for the metabolic action to develop (11, 50). However a recent study indicates that K^+, released by contracting muscle fibers, could also mediate an early dilatory effect (2), which leaves the issue unsettled.

Based on the similarity in the rapid hyperemic responses that have been reported in different preparations in response to short lasting muscle contractions and mechanical stimuli (6, 8, 10, 18, 24, 33, 38, 47) we hypothesized that a single mechano-sensitive mechanism, activated by changes in transmural pressure, could underlie the different responses. Moreover, since it has been proposed that this rapid hyperemia is functionally meant to provide a prompt increase in muscle perfusion at the onset of exercise (9), we aimed to investigate whether this reactivity to the mechanical stimulus were differently exhibited by muscular and cutaneous vascular beds.

To test these hypotheses, the rapid hyperemia induced by the following manoeuvres, i.e., spontaneous muscle contraction, external compression of the muscle, stretch of the passive muscle

and occlusion of the relevant artery, is elicited and compared in the same experimental model. In addition the tissue-specificity of this vascular responsiveness is investigated in arteries exclusively supplying skin or muscle.

The study is based on a recently developed rabbit experimental model (37) that allows continuous monitoring of muscle blood flow from the masseteric branch of the facial artery, which exclusively supplies the anterior portion of the masseter muscle. This muscle exhibits spontaneous isolated short bursts of activity producing a transient hyperemia (35) thus offering an ideal model to investigate the contraction-induced rapid hyperemia. In addition, the superficial localization of both the masseter muscle and the masseteric artery allows for convenient application of external stimuli such as muscle compression and artery occlusion.

The obtained results strongly support the notion that the contraction-induced rapid hyperemia can be largely mediated by mechano-sensitive dilatory mechanisms.

Methods

Ethical approval

The study was performed at the University of Torino in accordance with the principles of laboratory animal care. Purposes and protocols were approved by the Ethical Committee for Animal Experiments at the University of Torino.

Experiments were carried out on 12 anesthetized rabbits. The rabbits were previously implanted with chronic probes and in five of them recordings were also collected in the awake condition, the results being separately presented.

Surgical procedures

Twelve male European rabbits (Oryctolagus cuniculus) weighing between 2.8 and 3.3 kg were anesthetized with ketamine (Ketalar, Parke-Davis) and xylazine (Rompun, Bayer) at a dose of 20

and 5.5 mg/kg, respectively, injected into the marginal ear vein. Surgical anesthesia was subsequently maintained by continuous i.v. infusion of the same drugs (infusion pump Terumo Europe STC-521, Leuven, Belgium).

The surgery was performed in sterile conditions. The masseteric artery was isolated just after branching from the facial artery and accommodated into a perivascular flow probe (model 0.7PSB, Transonic Systems Inc, Itaha, NY, USA), adequate for vessels of diameter < 0.7 mm. The masseteric artery, which exclusively supplies the rostral portion of the masseter muscle, was isolated medially to the mandibular margin, immediately after its branching from the facial artery (37). The probe wire was tunneled below the platysma to the angle of the mandible and then subcutaneously around the neck. Particular attention was paid to avoid torsion of the cable that would have been transmitted by the probe to the vessel and cause interruption of blood flow. The small probe connector was left protruding from the dorsal cervical region, for subsequent connection to the flow meter, and sutured to the skin by means of a Delrin skin holder (Transonic Systems Inc.).

In five rabbits additional blood flow probes were implanted around the facial artery (model 1PSB Transonic Systems Inc, Itaha, NY, USA), proximal to the origin of the masseteric artery (35) and around the central ear artery (model 1PSL Transonic System Inc. Itaha, NY,USA), which supplies an almost exclusively cutaneous vascular bed (51).

A telemetric blood pressure transducer (TA11PA-D70, DSI USA) was implanted for continuous monitoring of arterial blood pressure (ABP). The catheter was inserted through the right femoral artery into the abdominal aorta while the transmitter was located in a subcutaneous abdominal pouch (35).

After surgery all animals underwent analgesic (Finadyne, Schering-Plough, 2 mg/kg i.m.) and antibiotic (Rubrocillin retard, Intervet, 0.1 ml/kg i.m.) treatment for the following 2 and 7 days, respectively.

After 7-10 days from surgery the animal was re-anesthetized as described above, the trachea was cannulated and the head was fixed in a stereotaxic frame by screws implanted in the frontal bone. In 5 rabbits a catheter was inserted in the left femoral vein for drug administration. In 4 animals electromyographic (EMG) activity of the masseter muscle was recorded ipsilaterally to the masseteric artery under investigation. EMG was detected by means of hooked copper wires, teflon-insulated except for their tips, inserted through a thin needle into the anterior portion of the muscle. The experimental procedures started after stabilization of the hemodynamic variables, about 1 h after the surgical preparation was completed.

Experimental protocol

The responses to the following stimuli were investigated: i) brief spontaneous contractions (BSC) of the masseter muscle, occurring in the absence of any external stimulus, ii) artery occlusion (AO), iii) muscle compression (MC) and iv) muscle stretch (MS) (Fig. 1).

Artery occlusions of short duration (about 1 s) were performed by the experimenter by means of a moderate compression of the skin against the inferior margin of the mandible at the point where the masseteric artery passes over the bone (Fig 1). AO lasting 1 s were later selected for analysis. Effectiveness of the maneuver was confirmed by the interruption of the blood flow in the recording. Muscle compression was performed by means of a shaker (model V2, Data Physics corporation, USA) driven by a computer-generated trapezoidal signal (rise time: 0.1 s; plateau: 1s; return: 0.1s, delivered by a1401 micro board, CED, UK). The shaker was armed with a semi-spherical tip (radius of 1 cm), having a linear excursion of 3-5 mm. This device allowed us to perform repeatable compressions of the cheek at the level of the anterior portion of the masseter muscle. Preliminary experiments showed that the amplitude of the hemodynamic response to MC has a limited dependence on the exerted force, in the range 0.9 – 3.6 N and that the responses are highly reproducible (manuscript in preparation). Muscle stretch was performed by manually

lowering the mandible by a fixed amount of 5-7 mm and maintaining the masseter muscle stretched for 1-2 s before returning the mandible to the resting position.

Artery occlusion was also operated both on the central ear artery, by manually compressing the vessel just distally to the flow probe, and on the facial artery, by manually exerting a pressure against the inferior margin of the mandible (Fig 1). Mechanical stimuli (AO, MC and MS) were provided in randomized order, separated by resting intervals of at least 2 min.

Vascular responses to MS were tested before and after administration of the neuromuscular blocking agent succinylcholine chloride (Sigma, Steinhein, Germany; 200-400 mg/kg, 3 animals). During neuromuscular blockade the animal was artificially ventilated so as to maintain the end-tidal CO_2 concentration at the pre-curarization level (in spontaneous breathing), measured by ossi-capnometer (Engstrom, Eliza Duo, Sweden). Effectiveness of neuromuscular blockade was assessed by monitoring the extensor movement of the paw elicited by electrical stimulation of the peripheral stump of the tibialis nerve.

At the end of the recording session the animal was sacrificed with an i.v. injection of a lethal dose of anesthetic.

Recording from conscious animals

Most of the recordings in awake animals concerned the investigation of hemodynamic responses to acute stress stimuli (36) that are not considered in the present study. Only recordings related to simultaneous monitoring of MaBF and EMG in resting conditions (5 animals) are included in the present study.

Measurements in awake animals started 5-7 days after implant of chronic probes and were repeated in subsequent days.

The animal was kept in a large box (50-36-20 cm) and familiarized with the stimuli performed by the experimenters, including touching the head and exerting light pressures with the fingers on the cheeks and mandible.

During the recording session the animal was let free to rest, move and eat in the box. Blood flow in masseteric artery was continuously recorded during rest and while handling the animal to provide different vascular stimuli: AO could be performed as described above while MC was performed by bilaterally compressing the cheeks with two fingers (thumb and index finger) at the level of the anterior portion of the masseter muscle, for a duration of about 1s.

Trials were considered valid when the maneuvers did not produce stress, as assessed by the absence of relevant changes in ABP, heart rate and blood flow in the artery under study (36).

Data acquisition and processing

During recording sessions, the arterial blood pressure signals were radio-transmitted from the implanted transducer to a nearby located antenna and reconverted to an analogue voltage signal (TA11PA-D70, DSI USA) while the flow probes were connected to the flow meter (2-channels TS420, Transonics, USA) via 1.5 m extension cables. Bipolar EMG recordings were amplified (P511 Grass, USA) and band-pass filtered (100-2000 Hz). All signals were then digitally sampled (1401micro, CED, UK) (sampling rate: 200 Hz for blood flow and arterial blood pressure, 5 kHz for EMG) and continuously acquired and stored on a personal computer. Acquisition and off-line processing were performed with Spike2 (CED, UK). Simple algorithms were implemented in the Spike2 script language aimed at identifying single cardiac cycles (based on systolic peak detection on the ABP signal) from which time averages of the different signals (one value per cardiac cycle) were computed, unless otherwise specified. Hyperemic responses were normalized with respect to their pre-stimulus value. Average responses to the different stimuli (BSC, AO, MC, MS) were obtained for each animal (n trials: 5-10) and then across animals. The average blood flow trace was computed after aligning single trials with respect to the start of the steep rising phase of hyperemic

responses, unless otherwise specified. In order to characterize and compare these hyperemic responses the following variables were calculated: the maximum blood flow increase (peak amplitude), the time elapsed from the beginning of the response to the flow peak (time-to-peak) and the time required to return from the peak to the mid value between peak and control level (half-return time).

All values are reported as mean ± standard deviation.

The comparison of responses to the different stimuli in anesthetized animals was performed by 1-way multi-variate ANOVA for the 3 variables: peak amplitude, time-to-peak and half return time. The comparison of BSC responses in anesthetized and conscious animals was performed by a 1-way multi-variate ANOVA. The comparisons between AO responses in masseteric, ear and facial arteries was tested by a 1-way ANOVA. Accepted significance level: $P < 0.05$.

Results

Hyperemic responses in anesthetized animals

In the anesthetized rabbit BSCs of the masseter muscle were often observed. They are characterized by a short EMG activation immediately followed by a marked increase in MaBF, one example being shown in Fig. 2A. This motor pattern, which is spontaneously exhibited at intervals ranging between 10 s to several minutes, appears to be rather stereotyped also across different animals. Fig. 2B reports the average curve obtained from 4 animals in which EMG and MaBF were simultaneously recorded. Note that the EMG curve well preserves its brief and sharp activation pattern. The fast transients are magnified in Fig. 2C. It can be observed that the latency between the beginning of the EMG burst and the increase in blood flow is in the order of 300-400 ms and that a brief reduction in blood flow occurs during muscle activation, preceding the immediate blood flow increase.

Very similar hyperemic responses were evoked by AO, MC and by MS. The responses developed immediately after the release of the stimulus and terminated within 10-20 s. Responses to BSC, AO, MC and MS were collected from 8 rabbits and the average curves are superimposed for comparison in Fig. 3A after normalization with respect to the control (pre-stimulus) value. Average parameters are as follows, BSC: peak amplitude = 394 ± 82 % (1.15 ± 0.54 ml/min), time-to-peak = 1.8± 0.8 s, half-return time = 6.1 ± 1.0 s; AO: peak amplitude = 426 ± 158 % (0.95 ± 0.50 ml/min), time-to-peak = 1.9 ± 0.9 s, half-return time = 7.5 ± 2.7 s; MC: peak amplitude = 469 ± 200 % (0.87 ± 0.40 ml/min), time-to-peak = 1.8 ±0.6 s, half-return time = 8.0 ± 3.7 s; MS: peak amplitude = 384 ± 171 % (0.75 ± 0.52 ml/min), time-to-peak = 2.4 ± 0.7 s, half-return time = 7.4 ± 1.6 s. None of the parameters was significantly dependent on the type of stimulus ($p=0.23$).

None of the stimuli affected arterial blood pressure. Thus, the relative changes in MaBF depicted in Fig. 3 can be read in terms of changes in vascular conductance as well. For the sake of clarity average curves are also reported in non-normalized form in Fig. 3B.

Response to muscle stretch after neuro-muscular blockade

Reflex activation of the masseter muscle was observed to occur in response to MS. In order to ascertain the role of muscle contraction in the MS-induced hyperemia, in 3 animals the maneuver was performed before and during transient muscle paralysis produced by systemic administration of succinylcholine. The effectiveness of neuro-muscular blockade was confirmed by the disappearance of reflexly-activated EMG activity. In all tested animals the rapid increase in blood flow occurring at the time of muscle release (peak amplitude: 394 ± 52 %) was still present after neuromuscular blockade (465± 77 %).

Hyperemic responses in conscious animals

Basal MaBF in the conscious rabbit ranged between 0.1 and 0.3 ml/min and always increased in connection with the activity of the masseter muscle, as detected by EMG. The recordings reported

277 in Fig. 4 show the hyperemic events associated with one typical BSC and with a sustained
278 masticatory activity, clearly indicated by EMG. Brief spontaneous contractions occurred
279 occasionally during rest; they evoked a hyperemic response characterized by: peak amplitude = 382
280 ± 119 %, time-to-peak = 1.8 ± 0.8 s and half-return time = 4.4 ± 1.5 (n=5) that was not statistically
281 different from the BSC response recorded in anesthetized animals. Details of the rapid hyperemia at
282 the onset of muscle activity are presented in Fig. 4a and b. In Fig. 4a it can be observed that the
283 latency of blood flow increase, with respect to the beginning of EMG activation, can be as low as
284 0.4 s. The recordings also clearly evidence the transient flow decrease or even flow reversal that
285 occurs during the contraction.
286 Hemodynamic response to mechanical stimuli were also evoked in awake rabbits and confirmed
287 that the transient hyperemia produced by BSC can be reproduced by AO and MC. A representative
288 recording is presented in Fig. 5 showing MaBF increases of comparable magnitude in the response
289 to BSC, AO and MC evoked in the same awake rabbit.
290
291 *Hyperemic responses in facial and ear arteries*
292 In order to ascertain the role of cutaneous circulation in mechanically-induced hyperemia the
293 occurrence of the myogenic response was specifically tested in the facial artery and in the central
294 ear artery, which supply a mostly- and exclusively-cutaneous vascular bed, respectively. In this
295 respect, note that occlusion of the facial artery is performed distally to the branching point of the
296 masseteric artery (as indicated by the open triangle in Fig. 1) which means that the mechanical
297 stimulus did not affect the vascular areas of the masseter and pterygoid muscles. Basal blood flow
298 ranged, in different animals and experimental conditions, between 4 and 12 ml/min in the facial
299 artery and between 0.5 and 8 ml/min in the ear artery. As shown in Fig. 6, the blood flow response
300 to 1-s artery occlusion was markedly smaller for the facial (peak amplitude: 83 ± 18 %) and for the
301 ear artery (68 ± 20 %) as compared to the masseteric artery (521 ± 105 %), in the same animals
302 ($p<0.01$, n=5).

Discussion:

The rapid contraction-induced hyperemia has been investigated *in vivo,* in an experimental model characterized by two innovative features: i) blood flow is recorded from an artery supplying exclusively skeletal muscle tissue, which allows to quantify the real changes in muscle perfusion, without the confounding action of possible changes in cutaneous circulation; ii) EMG activity is simultaneously recorded from the relevant muscle, which allows to accurately analyze the time relationship between the onset of motor activity and the hyperemic response.

For the first time, the hyperemia evoked by brief spontaneous muscle contractions was shown to develop with a latency as low as 300-400 ms from the beginning of the EMG activation and to be closely reproduced by brief occlusions of the artery supplying the muscle, as well as by other mechanical stimuli. In addition, cutaneous vascular beds supplied by facial and ear arteries exhibited a much lower mechano-sensitivity than the muscular vascular bed supplied the masseteric artery.

The rapid hyperemic response to brief spontaneous contractions

The rapid increase in blood flow or arteriolar diameter in response to spontaneous or stimulated contractions was investigated by several authors in both animal and human models (2, 22, 24, 31, 33, 41, 47). However, with a few exceptions in which skinned hind limbs or isolated muscle preparations were investigated (e.g. Mohrman and Sparks (31)), in most studies blood flow was measured from large arteries, supplying both cutaneous and muscular vascular beds. As a consequence, the increase of muscle blood flow was “diluted” with unaffected cutaneous blood flow, resulting in attenuated blood flow changes in the artery under study. In addition, the previously employed experimental models did not allow to investigate the flow changes occurring within the first second after the contraction and did not relate the hyperemic response to the onset of EMG activity in the relevant muscle. This may be partly due to the fact that in many studies muscle

329 contraction was obtained by electrical stimulation (31-33, 47). In these studies the hyperemia was
330 reported to develop within 1 s from the end of the 1-s lasting stimulation, i.e., within 2 s from the
331 onset of muscle contraction. Human studies based on voluntary muscle contraction (1-s lasting
332 isometric handgrip) provided a similar indication (19, 24). In the present study, the possibility to
333 monitor blood flow in a purely muscular artery during stereotyped BSCs allowed to detect a sharp
334 hyperemic response exhibiting a 5-fold blood flow increase in less than 2 s and developing with a
335 delay as low as 300-400 ms from the onset of EMG activity and with no detectable latency from the
336 end of the contraction (Fig. 2C). Responses with similar magnitude and time course were observed
337 in awake animals (Fig. 5A), supporting the notion that vascular reactivity is not affected by the
338 anesthesia.
339
340 *Possible role of metabolic mechanisms in the response to brief spontaneous contractions.*

341 The possibility that metabolic signals could take part to the rapid dilatation at the onset of exercise
342 has long been discussed (7, 16, 45, 50). By administering different dilatory metabolites to isolated
343 skeletal muscle arterioles it was shown that at least 4 s are required to elicit dilatation (50). More
344 recently Armstrong et al. (2) suggested that potassium released by the excitation of muscle fibers
345 could play a major role in the early dilatory process, based on the observation that the dilatation
346 (evaluated at 4 s after muscle contraction) was markedly reduced by application of various blockers
347 of the K^+ signalling pathway. However, these data have been questioned (9, 23) on the basis of the
348 following two facts: i) some of the pharmacological interventions weakened the contraction of
349 skeletal muscle fibers, thus also attenuating the mechanical action exerted on blood vessels ii)
350 voltage-gated K^+ channels are also implicated in the myogenic response (15), thus their blockade
351 might have impaired the myogenic reactivity of the vascular network. Therefore, it is argued that
352 production and diffusion of vasodilatory metabolites cannot account for the rapid onset of
353 vasodilation with contractions (9), although this remains to be determined

Based on the present results several lines of evidence argue against a role of metabolic signals in the response to BSC: i) the hyperemic response develops as early as 300-400 ms from the onset of EMG activation, with a time-to-peak of 1.8 s, i.e. a time course that, on the basis of the available knowledge is not compatible with the metabolic dilatation, ii) the hyperemic response to BSC is replicated by mechanical stimuli, such as AO and MC, that do not activate metabolic pathways; iii) the hyperemic response to MS was not prevented by blockade of the reflexly-induced muscle contraction.

Muscle pump and veno-arteriolar reflex

Several studies have compared the hyperemia induced by a single contraction with that evoked by a brief increase in external pressure (6, 24, 31). While this approach provided good evidence of a mechanically-dependent hyperemia, it did not help in understanding the possible contribution by the "muscle pump" mechanism (26, 45). In fact, both maneuvers squeeze and empty intramuscular vessels thus reducing venous blood pressure and increasing perfusion pressure (muscle pump). In addition the reduced venous pressure could stimulate a rapid dilatation through the venous-arteriolar reflex (44). When external compression is exerted on an intact limb, these actions are also exerted on cutaneous layers, as well as to muscles not involved in the contraction, which complicates the comparison of the response with the contraction-induced hyperemia.

In the present study we showed, in both anesthetized and conscious animals, that the rapid hyperaemia evoked by spontaneous contractions and external compressions is also reproduced by short lasting AO. Occlusion of the masseteric artery exclusively affects the musculo-vascular bed under investigation, with no involvement of the cutaneous layer and no activation of muscle pump mechanisms. In particular, due to the absence of mechanical emptying of the venous compartment and to the dampening effect of upstream vascular districts, the drop in venous pressure is expected to be smaller during AO then during BSC and MC. This suggests that the involvement of the

muscle pump mechanism and of the veno-arteriolar reflex is not a necessary condition for the development of a rapid hyperemia such as is produced by BSC and single short-lasting MC.

Mechano-sensitivity of the vascular network

What BSC, MC and AO have in common is that they all produce a transient transmural pressure decrease in the vascular network of the muscle. However, while BSC and MC potentially activate also other mechanisms, such as the release of dilatory metabolites and the muscle pump as discussed above, the effects of AO are almost exclusively limited to the decrease in transmural pressure in the vascular bed located down stream to the occlusion site. Thus, the observation that the response to AO mimics the response to BSC and MC strongly suggests that the reduction in transmural pressure is the relevant stimulus for the rapid hyperemia.

On the other hand, the same rapid hyperemic response was produced by MS in both paralyzed and non-paralyzed muscles. Whether MS also resulted in increased intramuscular pressure and reduced vessels' transmural pressure is not known, although it produced, like BSC, a slight decrease in blood flow immediately prior to the hyperemia (Fig 3), indicating some compression of the vascular network.This possibility is supported by observation of a slight increase in intramuscular pressure of the quadriceps during passive flexion of the knee (34). In addition blood vessels may be stretched/squeezed together with muscle fibers and these actions may be as effective as the decrease in transmural pressure in inducing a dilatory response. Thus, it seems reasonable to hypothesize that MS activates the same mechano-sensitive pathways as the other stimuli. This observation fits with several reports of hyperemia induced by passive limb movement (34, 43, 48).

Rapid dilatation and the myogenic response

As early as in 1974, Mohrman & Sparks (31) put forward the hypothesis that the decrease in transmural pressure occurring during a brief contraction could produce an active dilatation, according to the myogenic response.

406 In fact, the myogenic response, also called Bayliss effect, is usually described as the
407 increase/decrease in vessel tone in response to the increase/decrease of transmural pressure (3, 15,
408 21, 52). It has been well characterized *in vitro*, in cannulated arterial segments subjected to
409 sustained step changes in transmural pressure (13, 15, 21), while the rapid response to short lasting
410 changes has been comparatively less investigated. This is surprising if we consider that the first
411 observation of the phenomenon by Sir William Bayliss was in fact a rapid blood flow increase in
412 the hind limbs in response to a brief occlusion of the abdominal aorta (3). The existence of a rapid
413 myogenic constriction (28, 29, 49) and dilatation (5, 38) has then been documented in different
414 preparations.
415 However, whether the rapid dilatation evoked by transmural pressure changes in muscle blood
416 vessels is exclusively myogenic in nature is still debated. In particular, in isolated muscle arterioles
417 it has been recently shown that endothelium-mediated dilatation can develop as early as 1-2 s from
418 a step increase in flow delivered in the absence of transmural pressure changes (4). This mechanism
419 may have contributed to the hyperemic responses described in the present study. In addition it has
420 been hypothesized that increased NO release and dilatation could also result from direct mechanical
421 deformation of the endothelium (14, 25). The rapid dilatation induced by external compression of
422 isolated muscle feed arteries was shown to be partly dependent on endothelium integrity (10). In
423 humans, blockade of NO-synthesis by l-NMMA markedly reduced the rapid hyperaemia elicited by
424 passive movements (43) while it did not substantially affect the hyperemic response to 1-s lasting
425 compressions of the forearm (6). In the present study the presence of an endothelium-mediated
426 component in the observed hyperemic responses was not investigated and cannot be excluded. For
427 this reason, the term “rapid *myogenic* response” may be inappropriate and the alternative notation
428 “Bayliss effect” is here preferred. Further studies are required to clarify what is the role played by
429 the endothelium.
430

In humans, Kirby et al. (24) evidenced a temporal dissociation between the hyperemic responses to forearm compression and to muscle contraction, the former peaking earlier (1-2 cardiac cycles) than the latter (4 cardiac cycles). On this basis they left the possibility open for other mechanisms to contribute to the contraction-induced rapid dilatation (24). Our results did not evidence the same temporal dissociation: the responses to all types of stimuli employed peaked between 1.8 and 2.4 s (Fig. 3) and were not significantly different; however, many factors may justify small differences between the responses. In particular, in their study, blood flow measurement included adipose and cutaneous blood flow, although the influence of the latter was minimized by skin cooling. It is likely that this might have differently affected the response to spontaneous contraction and to cuff compression of the forearm, also considering the potentially different vascular mechano-sensitivity of the different tissues, as observed in the present study for skin and muscle blood flow.

Muscle vs. cutaneous vascular reactivity

The myogenic response is known to be differently expressed by vessels belonging to different tissues and organs (21, 30), as well as by the different segments of the same vascular bed (13, 17). In this respect, a prominent myogenic reactivity is described for arterial and arteriolar segments of brain, skeletal muscle (5) and kidney while no explicit reference is usually made to skin blood vessels (30, 39, 40). To our knowledge, only few studies described the occurrence of a *slow* myogenic response in cutaneous vascular beds (13, 20, 42) developing in 1-2 min upon sustained changes in transmural pressure, while the *rapid* component has never been investigated.

In the present study both the exclusively cutaneous ear artery and the mostly cutaneous facial artery showed only a slight blood flow response to AO (Fig. 6). It is tempting to speculate that a high mechano-sensitivity is a prominent characteristics of muscular vessels that is not shared by cutaneous vessels. Alternatively, it could partly be attributed to a greater dilatory reserve of resting muscle, as compared to cutaneous tissues. Irrespective of the underlying mechanisms, the cutaneous

456 vascular bed exhibited a much lower response to mechanical stimulation, as compared to the
457 muscular bed, in physiological conditions.
458
459 *Concluding remarks*
460 In summary, several lines of evidence were gathered indicating that a mechano-sensitive dilatation
461 responding to changes in transmural pressure mediates the rapid hyperemia at the onset of exercise:
462 i) the contraction-induced hyperemia occurs as early as 300-400 ms from the onset of EMG, which
463 makes highly unlikely a role of diffusible metabolic signals; ii) contraction-induced hyperemia is
464 qualitatively and quantitatively mimicked by the response to mechanical stimuli that do not affect
465 metabolic activity, i.e., AO, MC and MS in paralysed muscles; and do not involve the muscle pump
466 nor the veno-arteriolar reflex, i.e., AO. Thus, the rapid hyperemic responses to these different
467 stimuli are all consistent with the Bayliss effect, while the relative contribution of myogenic and
468 endothelial mechanisms remains to be ascertained.
469 These rapid hyperemic events, observed in the rabbit masseter muscle, are strikingly similar to
470 those reported in response to artery occlusion, muscle compression, electrically-evoked and
471 spontaneous contractions in different muscle groups and animal species (6, 8, 10, 18, 24, 33, 38,
472 47). This indicates that such rapid dilatation is a characteristic of the musculo-vascular network that
473 is highly preserved across different species and that the masseteric artery of the rabbit may be a
474 good model for its investigation.
475 The preliminary observation that the rapid dilatation is only expressed by muscular and not by
476 cutaneous vascular networks supports the hypothesis that it is meant to subserve a feedforward
477 control of muscle blood flow (9). Based on this mechanism, a prompt increase in blood supply is
478 granted to the muscle at the very onset of exercise, before slower metabolic feedback mechanisms
479 accurately match perfusion to the actual metabolic demand.
480
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Author contributions

Conception and design of the experiments: SR.

Collection, analysis and interpretation of data: MT and MM.

Drafting the article or revising it critically for important intellectual content: MT and SR.

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626

627 **Figure legends**

628

629 Fig 1: Anatomical scheme illustrating the masseteric branch of the facial artery and the location of
630 the perivascular blood flow probe. The different mechanical maneuvers used in this work are
631 indicated by dashed arrows: mandibular stretch (MS), muscle compression (MC) and artery
632 occlusion (AO). The closed and open triangles indicate the point where the masseteric and the facial
633 artery, respectively, are occluded.

634

635 FIG 2: Masseter artery blood flow (MaBF, grey line) and EMG activity of the masseter muscle
636 (EMG, black line) are shown during brief spontaneous contractions (BSC) of the masseter muscle
637 in anesthetized animals. A) original recordings of a single BSC; B) average curves (n = 4 animals);
638 C) time zoom of the fast transients in part B: note the short latency between the rapid dilatation and
639 the EMG burst, indicated by vertical dashed lines.
640 In B and C, the EMG signal was rectified and low pass filtered (moving average width: 20 ms),
641 MaBF was low-pass filtered (cut-off frequency:13 Hz) before averaging. The trigger signal used for
642 the average was based on the positive peak of the EMG signal. In C the thin grey line represents the
643 average curve obtained from unfiltered blood flow tracings. This overlaps the filtered MaBF (thick
644 grey line), indicating that the filtering process did not introduce time shifts.

645

646 FIG 3: Masseteric artery blood flow (MaBF) responses to different mechanical stimuli are
647 superimposed on the response to brief spontaneous contractions (BSC) reproduced from Fig. 2.
648 In A) Normalized responses expressed as % of control (pre-stimulus) value. B) Non normalized
649 resonses. AO= artery occlusion; MC= muscle compression; MS= muscle stretch; (n= 8 animals).

650

651

FIG 4: Simultaneous recording of blood flow from the masseteric artery (MaBF) and electro-myografic (EMG) activity of the masseter muscle in a conscious rabbit. Large increase in MaBF can be observed both during one brief spontaneous contraction (BSC) and during mastication of a carrot, as indicated. The phase of rapid increase in blood flow is magnified in insets a) and b) respectively. Note the short latency, below 400 ms, between the EMG activation and the increase in blood flow, preceded by a slight decrease (inset a).

FIG 5: Hyperemic responses evoked in an awake rabbit. A) brief spontaneous contraction; B) occlusion of the masseteric artery and C) compression of the masseter muscle.

FIG.6. Comparison of the myogenic responses of different vascular beds evoked by brief occlusion of the relevant arteries. Facial artery blood flow (FaBF, light grey) , masseteric artery blood flow (MaBF, black) and ear artery blood flow (EaBF, dark grey). Occlusion of the facial artery was performed distally to the origin of the masseteric branch (see Fig. 1, open triangle). Note the little responsiveness of blood flow to occlusion of the facial and ear arteries. Each curve is the average of responses collected from 5 animals)

Fig. 1

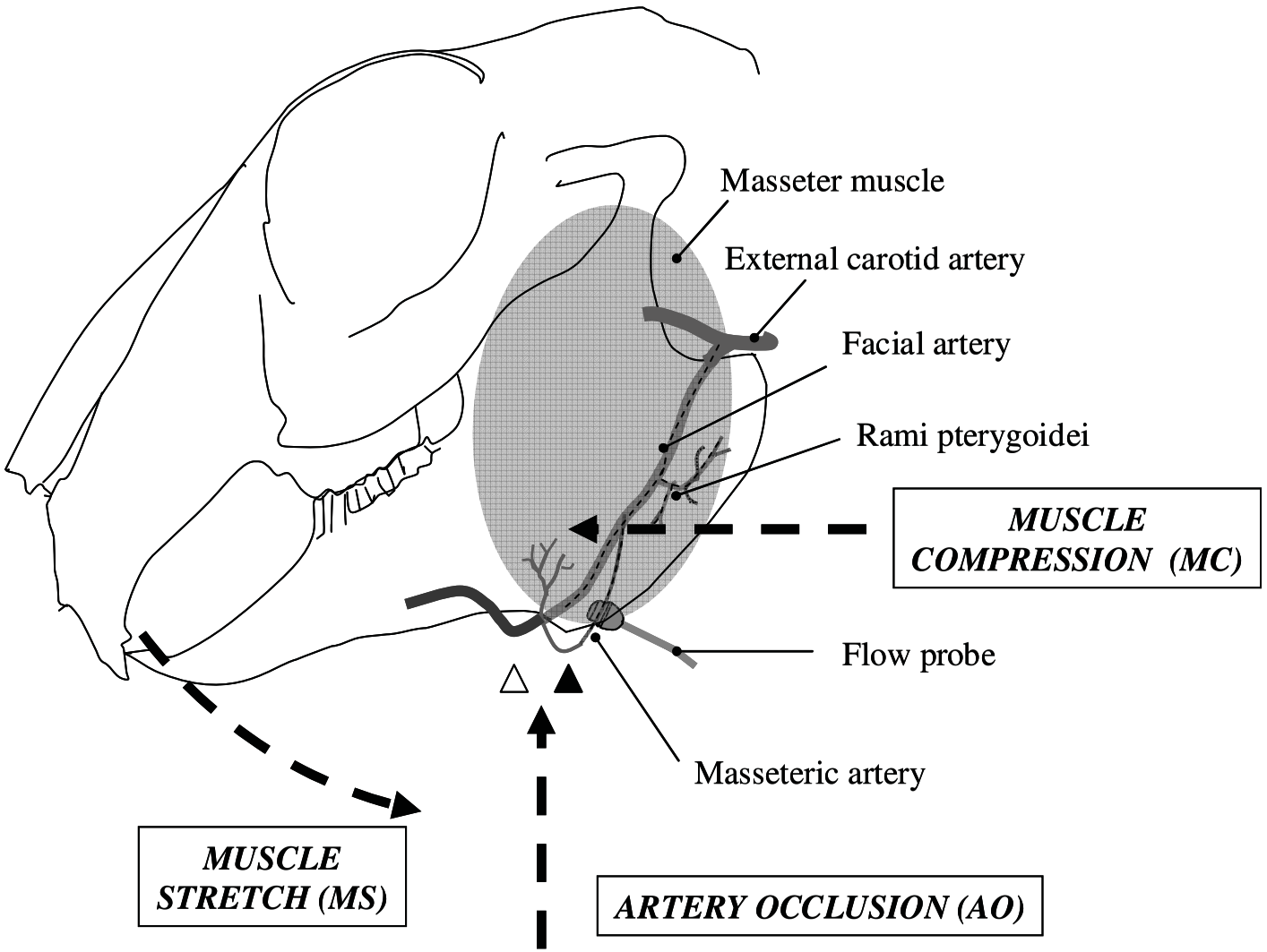
Masseter muscle
External carotid artery
Facial artery
Rami pterygoidei
MUSCLE COMPRESSION (MC)
Flow probe
Masseteric artery
MUSCLE STRETCH (MS)
ARTERY OCCLUSION (AO)

Fig. 2

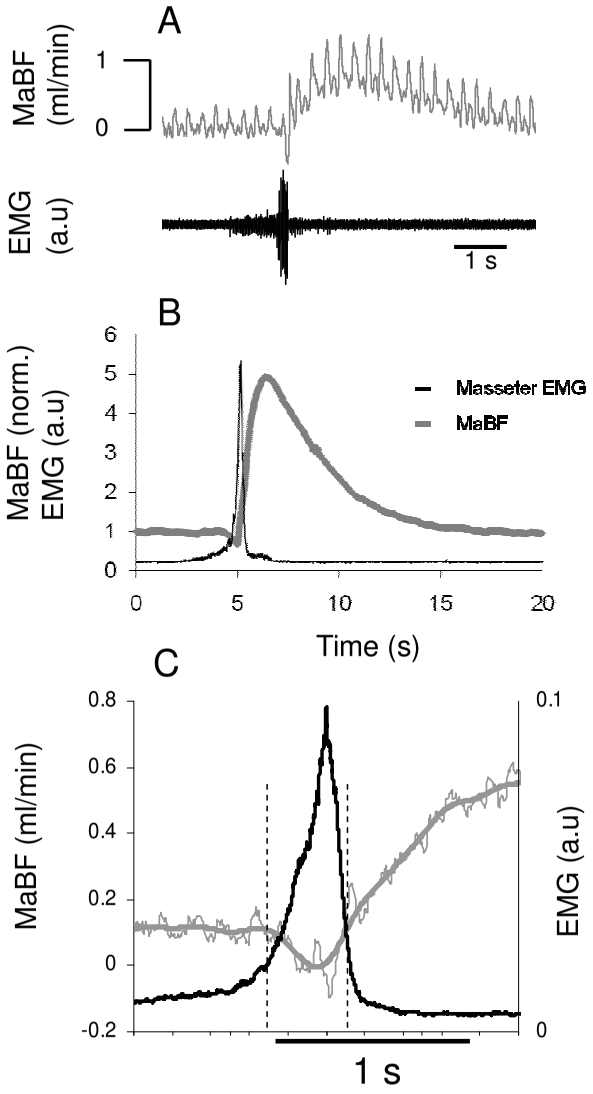
A
MaBF (ml/min)
1
0
EMG (a.u)
1 s
B
MaBF (norm.) EMG (a.u)
Masseter EMG
MaBF
Time (s)
C
MaBF (ml/min)
EMG (a.u)
1 s

Fig. 3

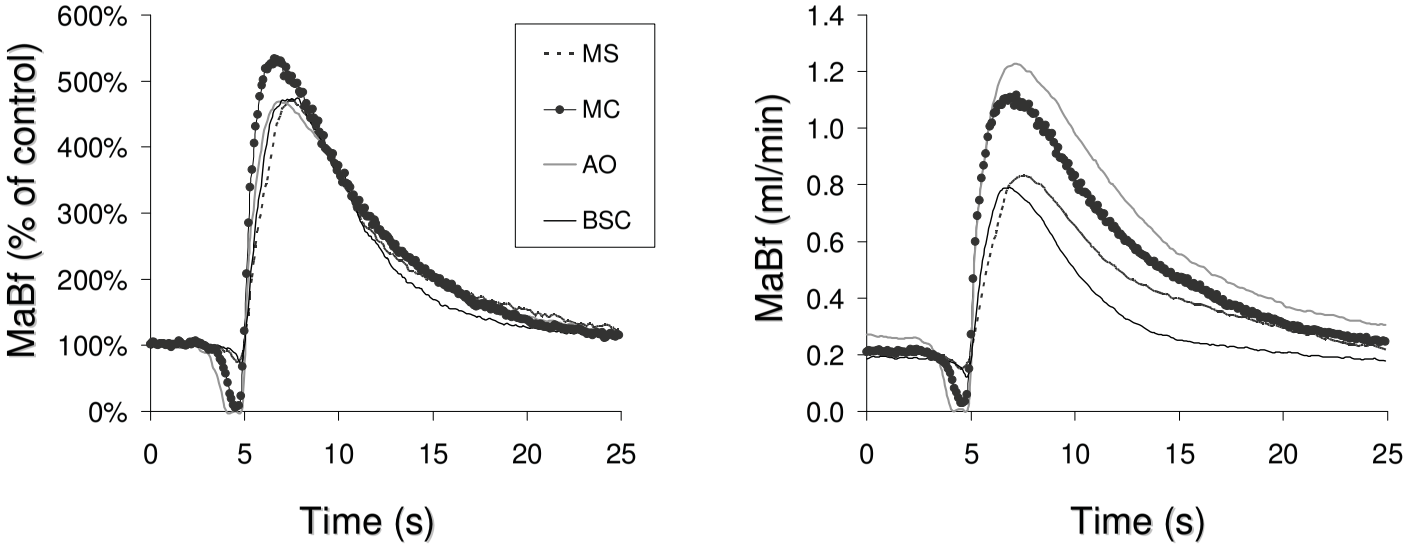
600%
500%
400%
300%
200%
100%
0%
MaBf (% of control)
MS
MC
AO
BSC
0
5
10
15
20
25
Time (s)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
MaBf (ml/min)
0
5
10
15
20
25
Time (s)

Fig. 4

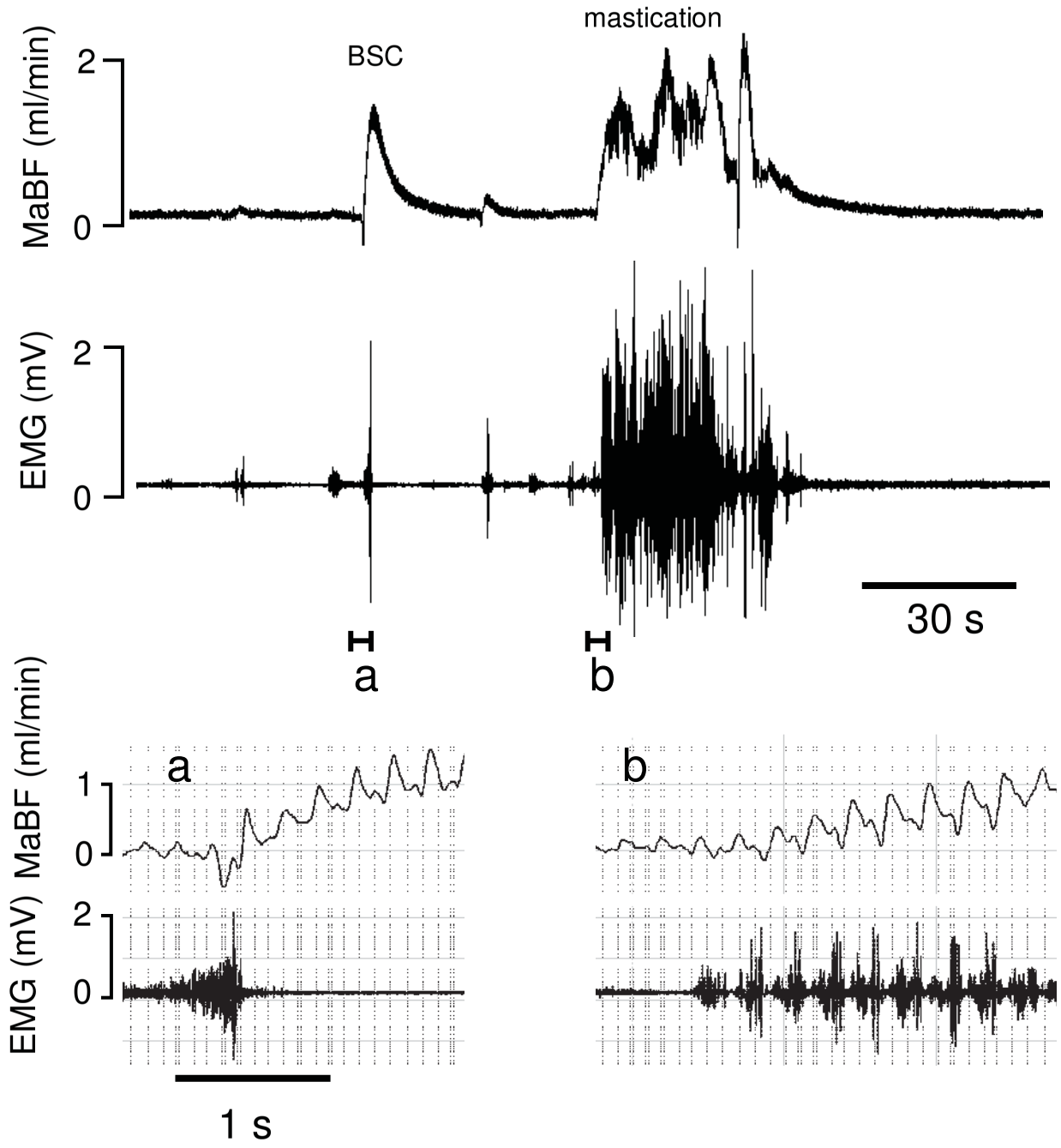
mastication
BSC
MaBF (ml/min)
2
0
EMG (mV)
2
0
30 s
a
b
a
b
MaBF (ml/min)
1
0
EMG (mV)
2
0
1 s

Fig. 5

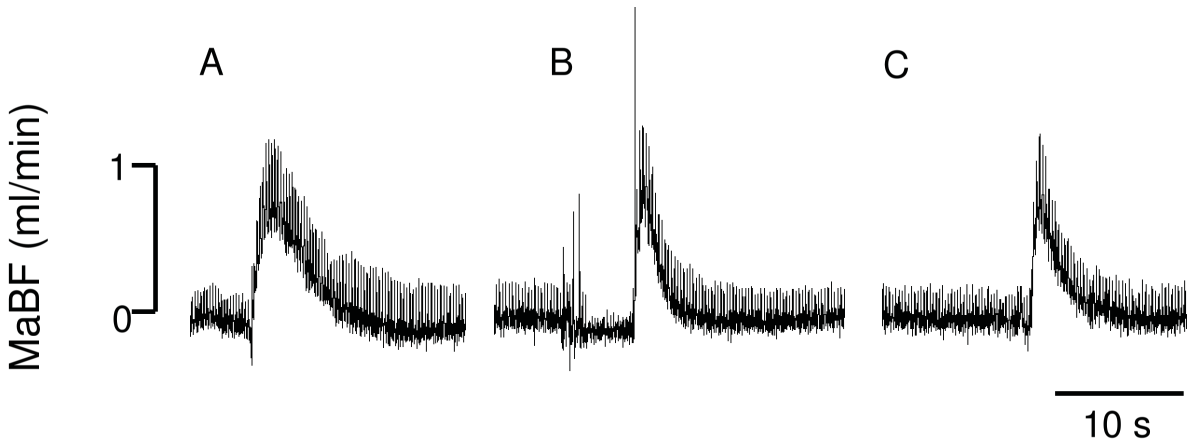

Fig. 6