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A model for investigating the control of muscle blood flow: the masseteric artery in conscious rabbits

S Roatta [1] , M Mohammed [1], M Turturici [1], L Milano [1] and M Passatore [1]

[1] Neuroscience Dept., Physiology Div., University of Torino Medical School, Torino, Italy

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Corresponding author:
Silvestro Roatta
Dip. Neuroscienze, Sez Fisiologia
c.so Raffaello 30, 10125 Torino, Italy
Tel.: +390116708485 Fax: +390116708174
Email: silvestro.roatta@unito.it

Abstract

The complex interplay of neural, metabolic, myogenic and mechanical mechanisms that regulate blood flow in skeletal muscle (MBF) is still incompletely understood. For the first time, a method is presented for high time-resolution recording of MBF from a purely muscular artery in physiological conditions. Ultrasound perivascular flow probes were implanted (n=15) mono- or bilaterally around the masseteric branch of the facial artery in 9 rabbits and tested up to 16 days after implant. Reliable and stable recordings were achieved in 50% of implants. Blood flow was observed to increase from a resting level of 0.2-0.3 ml/min up to 4.0-6.0 ml/min during spontaneous masticatory activity. In addition, within single masticatory cycles marked back flow transients could be observed (peak flow= -10 ml/min) during powerful masticatory strokes but not during mild mastication. The possibility to 1) surgically remove the sympathetic supply to the relevant vascular bed and to 2) bilaterally monitor the perfusion of masseter muscles thus allowing to use one side as control side for different types of interventions, make this model a useful tool for disentangling the different mechanisms involved in the control of MBF.

Keywords: muscle blood flow, Haemodynamics, Arterial blood flow, Animal model, Vascular Control, Ultrasound, hyperaemia, masseter muscle, conscious animal

1. Introduction

In spite of the large number of studies carried out over more than a century, the control of muscle blood flow is still incompletely understood (Saltin *et al.* 2007). The reason is related to the complex interplay between different regulatory actions, which include autonomic neural control (Thomas and Segal, 2004; Seals and Victor, 1991), metabolic (Thomas and Segal, 2004; Joyner and Wilkins, 2007), myogenic and endothelium-mediated mechanisms (Johnson, 1980; Mellander, 1989; Laughlin *et al.*, 1996), and the mechanical action exerted by muscle contraction on the vascular bed (Laughlin *et al.*, 1996).

Muscle blood flow (MBF) has been investigated in both animal models and humans with different techniques, each of them being characterized by specific advantages and limitations (Radegran, 1999). Techniques like thermo-dilution MRI and PET specifically measure muscle blood flow but do not grant a high temporal resolution. Eco-Doppler devices provide a high time resolution and are non invasive, however they provide an indirect measure of blood flow that is based on continuous measurement of blood velocity and cross-sectional area of the insonated vessel. Accuracy of this measure improves with vessel dimension, however large vessels supply mixed (musculo-cutaneous) vascular beds which brings some limitation to the assessment of MBF. Perivascular flow probes (electromagnetic or ultrasound flowmetry) give the most accurate blood flow measure at the highest time resolution. This invasive technique has been largely used for muscle blood flow investigations in a variety of animal models, however investigations were generally based on recordings from large limb arteries such as the femoral or iliac arteries, that supply also the skin. In some of these preparations the contribution of skin blood flow was however reduced or eliminated by skinning the limb (Celander and Folkow, 1953; Rowlands and Donald, 1968) or by ligating the paw (Celander, 1954).

It should be also considered that, while reduced and anesthetized preparations may be adequate for the investigation of local vascular mechanisms, anaesthesia is known to variably affect vascular tone, arterial blood pressure and autonomic reflexes (Rowlands and Donald, 1968; Nalivaiko *et al.*, 2005), which makes the conscious animal a preferred model in many respects. A number of studies with electromagnetic flow meters have been performed in conscious animals, e.g., for investigating autonomic vascular control under stressful conditions (Baccelli *et al.*, 1971; Mancia *et al.*, 1972; Yu and Blessing, 1997), however, also in these studies the effects on muscle blood flow were inferred from recordings from large arteries that also supply the skin.

To our knowledge there are no reports of recording blood flow from purely muscular arteries in conscious freely moving animals.

We here present the possibility to record MBF from the masseteric branch of the facial artery, a tiny vessel exclusively supplying the anterior portion of the masseter muscle. Recordings in conscious rabbits were achieved by means of chronic implants of perivascular ultrasound flow probes.

2. Methods

Experiments were carried out on 9 adult male European rabbits (Oryctolagus cuniculus) weighing between 2.8 and 3.3 kg, in accordance with the principles of laboratory animal care. Protocols were approved by the Ethical Committee for Animal Experiments at the University of Turin (Italy).

2.1 Surgical procedure

The rabbits were anesthetized with ketamine (Ketalar, Parke-Davis) and xylazine (Rompun, Bayer) at doses of 20 and 5.5 mg/kg, respectively, injected into the marginal ear vein; surgical anaesthesia was subsequently maintained by continuous i.v infusion of the same drugs.

Surgery was performed with the use of sterile procedures. A perivascular flow probe (model 0.7PSB, Transonic Systems Inc, Itaha, NY, USA) was mono- or bilaterally implanted on the masseteric artery (Ma), as depicted in Fig 1. The probe accommodates vessels of up to 0.7-mm diameter and the masseteric artery loosely fits into its J-shaped ultrasounds reflector.

The Ma, which supplies the rostral portion of the masseter muscle, was isolated medially to the mandibular margin, immediately after its branching from the facial artery, and inserted in the J-shaped probe (Fig. 2). This tiny artery is very sensitive to external pressures and can be occluded by any traction or torsion exerted by the probe, which may then result in the formation of permanent clots. At the insonation site the isolated masseteric artery is rather loosely connected to the underlying medial pterigoyd muscle, thus allowing for the perivascular insertion of the probe without causing excessive stretching of the vessel. Further wrapping of the probe with Dacron mesh (Ethicon, INC., Somerville N.J., USA), that is usually recommended for improving stability of chronic implants, was not performed to avoid vessel overstretching.

The probe was secured to the medial pterygoid muscle by means of a suture point on the cable in close proximity of the probe. The probe wire was tunnelled 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). A telemetric blood pressure transducer (TA11PA-D70, DSI USA) was also implanted, the catheter being inserted into the right femoral artery and the transmitter being located in a subcutaneous abdominal pouch.

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.) treatments.

For the recording sessions, performed on the conscious animal after recovery from surgery, the rabbit was kept in a box., Fresh food (carrots or slices of tomato) was given from the hands of the experimenters and masseteric artery blood flow (MaBF) was recorded during the mastication.

Electromyographic (EMG) activity from the masseteric muscle was occasionally recorded (6 rabbits) by means of hooked copper wires, Teflon-insulated except for their tips, acutely inserted in the muscle through a thin needle.

2.2 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, 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). The signals were then digitally sampled (1401micro, CED, UK) (sampling rate: 200 Hz for blood flow and 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.

Values are reported as mean ± standard deviation.

3. Results

Out of 15 implanted probes 3 failed to give any signal due to either clogging of the artery or to displacement of the probe. In successful implants 1-4 days (1.8± 0.9) were required to obtain stable flow recordings, i.e., without movement-related signal corruption due to loss of ultrasound conduction within the probe-vessel pathway. In 4 cases a premature interruption of the probe function occurred between 4 and 10 days after implant (7.0 ± 2.6 days), for the same reasons cited above.

In the remaining 8 implants blood flow recordings lasted till suppression of the animal (7-16 days after implant, average: 10.6±3.5) and provided good quality signals and stable flow resting levels (0.23+/- 0.10 ml/min) throughout the observation period.

In Fig. 3 a typical recording of MBF in masseteric arteries before and during masticatory activity is shown in a bilaterally implanted rabbit along with EMG activity of the left masseter muscle. In this occasion, blood flow exhibited a rather symmetrical pattern of gradual increase during mastication: from a resting level of about 0.3 ml/min to 6 ml/min. The high time resolution of the recording allows to evidence clear cardiac pulsatility in the resting condition (Fig. 3B, first 1.5 s). When rabbit was chewing hard food like carrots, as in this case, a large retrograde blood flow developed during the powerful muscle contractions that are evidenced by the EMG signal (Fig. 3C). The transient interruption of masticatory activity is associated with a further increase of blood flow (from 3.5 to 5.5 ml/min) (Fig. 3C, central part). Notably, the cardiac pulsatility in the Ma can only be detected during masticatory pauses, being otherwise heavily distorted by mechanical interference of muscle activity with blood flow (Fig. 3C).

4. Discussion:

For the first time a method is presented for continuous, high time resolution, recording of blood flow from an artery supplying exclusively skeletal muscle tissue. Although the success rate for the chronic probe implantation was not very high (about 50%), in many experiments stable flow recordings could be

maintained for several days, after recovery from the surgery. The possibility cannot be a priori excluded that, even in this cases, the perivascular flow probe might have affected blood flow in Ma, however few considerations can be made. First of all the surgical tissue perturbation is minimal given the relatively superficial location of the artery and the small size of the flow probe. After surgery the vessel-probe system is quickly embedded and stabilized by newly generated connective tissue, as observed *post-mortem*, which helps to prevent critical displacements of the probe that could occlude the vessel. Moreover, relative displacement between probe and tissues during movement is minimal since the probe is located close to the mandibular bone. In fact, even during powerful and large masticatory movements no flow interruption was observed; instead, a marked retrograde flow was measured, which rules out the possibility of artery occlusion during the movement. In addition, it should be also considered that ultrasound flow probes (at difference from electromagnetic probes) allow for lose accommodation of the vessel within the probe thus permitting the radial expansion of the vessel which may develop in feed arteries during exercise hyperemia. Finally, the similarity of the average values and waveform of blood flow signals, as well as of the time course of the response to chewing in simultaneously-recorded arteries further supports the notion that the probes were not substantially affecting the physiological conditions of blood perfusion and hemodynamics. Recording of blood flow from the masseteric artery thus allows to reliably quantify the flow changes occurring in the jaw muscle tissue, without the confounding effect of cutaneous circulation. The technique allows to accurately describe, in the awake animal, the details of the vascular changes occurring in the muscle, including the fast haemodynamic transients taking place at the beginning of muscle activity (Clifford and Tschakovsky, 2008) as well as the mechanical interference of dynamic muscle contraction with muscle perfusion (Laughlin, 1987). The role of the different mechanisms in functional hyperaemia is still subject of active research (Clifford and Tschakovsky, 2008; Joyner and Wilkins, 2007; Saltin, 2007). Although skin perfusion can be assumed to remain constant in certain circumstances (Rowlands and Donald, 1968), which allowed to gain much of the present knowledge on the control of MBF from recordings of musculo-cutaneous arteries (Baccelli *et al.*, 1971; Mancia *et al.*, 1972; Yu and Blessing, 1997; Radegran, 1999; Tschakovsky and Sheriff, 2004), direct MBF measurement is expected to provide further insights due to its higher potential in detecting and quantifying specific and subtle changes in muscle perfusion.
In addition, this model will prove a useful tool for investigating the interaction between local dilatory mechanisms and central neurovascular regulation (Thomas and Segal, 2004). The easy access to the cervical sympathetic trunk allows for local sympathetic decentralization or denervation by unilateral pre- or post-ganglionic sectioning of the trunk, respectively. By simultaneous recording from the two sides it is thus possible to compare vascular responses in the presence and in the absence of sympathetic control. Similarly, the effect of unilateral pharmacological or mechanical interventions can be investigated based on the comparison of the control side and the treated side. The comparison of MBF recordings from the two sides is facilitated by the fact that neural control and activation of masticatory muscles is symmetrical to a large extent (Jaberzadeh *et al.*, 2006; Lund and Kolta, 2006). This possibility was recently exploited in an investigation on stress-induced vascular responses in the facial artery of conscious rabbits, in which the

comparison of responses between the intact and sympathetically decentralized side allowed to discriminate neural and hormonal components in the vascular response to different stressors (Roatta *et al.*, 2009).

In conclusion, the present model allows for high time resolution recording of blood flow from a purely muscular artery in physiological conditions. Peculiar anatomical and functional features make it potentially helpful for disentangling the different mechanisms contributing to the regulation of muscle blood flow.

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Figure legends:

Fig 1: Anatomical scheme illustrating the site of implantation of the chronic probe on the masseteric branch of the facial artery. Dashed lines indicate the vessels located on the medial side of the mandibular bone.

Fig 2: Micrograph of the open window (inferior view of the mandible) showing the left masseteric artery (1) branching from the facial artery (2) and laying on the nano-probe (3). Connective tissue layer is pulled by surgical pliers (4). Calibration bar: 3 mm.

Fig 3: A) Simultaneous recording of blood flow from the right (RMa) and left (LMa) masseteric arteries and EMG activity from the left masseter muscle (LEMG) in the conscious rabbit chewing a carrot. The two portions of this chewing cycle marked as B and C under the EMG tracing are reproduced below, with an amplified time axis. B) In resting condition (left), baseline flow shows a clear and regular cardiac pulsatility (heart rate 240 bpm) and rapidly increases at the onset of muscle activity. C) individual masticatory cycles evidence the retrograde blood flow developing during the muscle contraction; the reappearance of cardiac pulsatility (heart rate: 255 bpm) can be observed during the transient interruption of the masticatory pattern. A line indicating the cycle-by-cycle mean value is superimposed on the flow signals.

Fig. 1

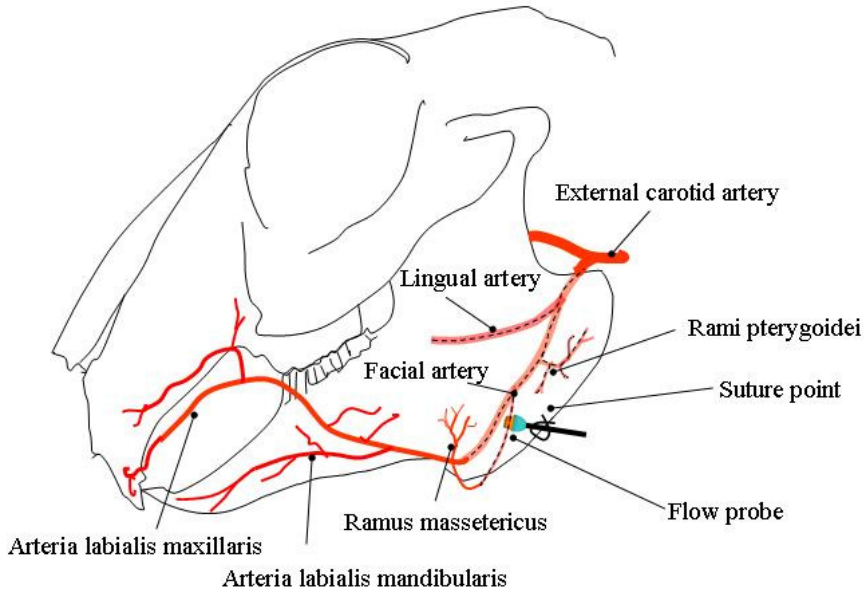
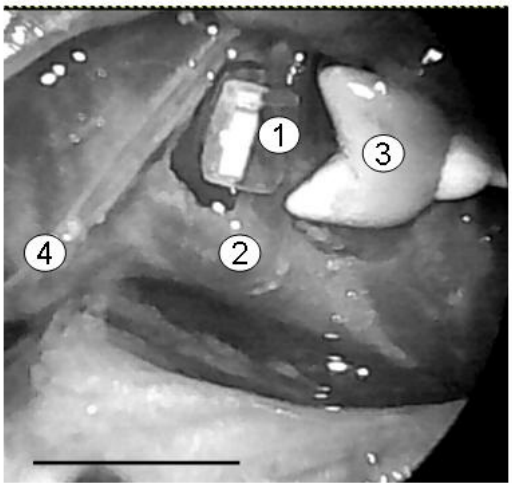

External carotid artery
Lingual artery
Rami pterygoidei
Facial artery
Suture point
Flow probe
Ramus massetericus
Arteria labialis maxillaris
Arteria labialis mandibularis

Fig. 2



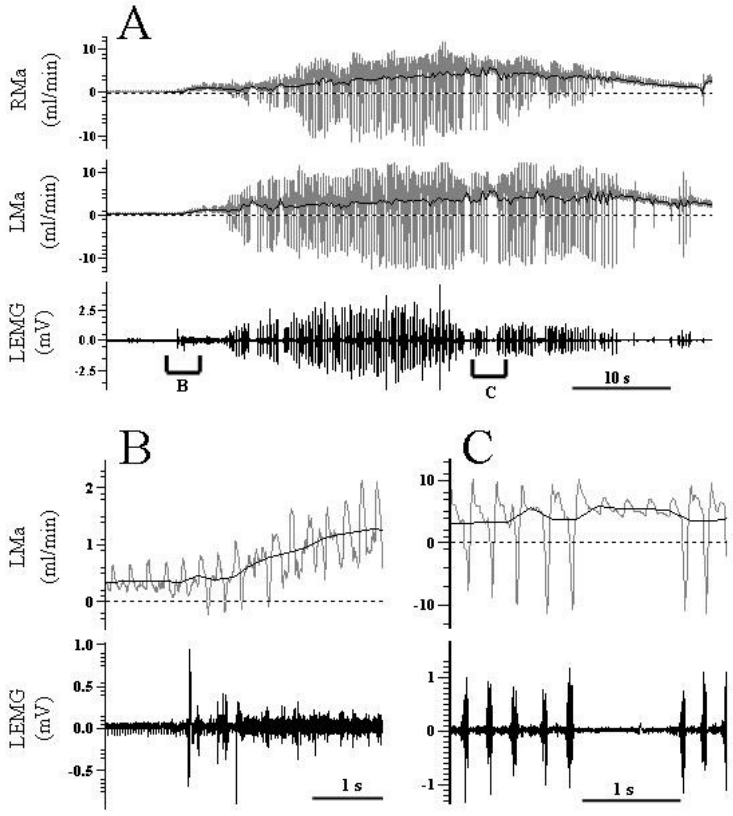


Fig. 3