ELSEVIER

Journal of Neuroscience Methods 104 (2001) 209–213

JOURNAL OF NEUROSCIENCE METHODS

www.elsevier.com/locate/jneumeth

Tail artery blood flow measured by chronically implanted Doppler ultrasonic probes in unrestrained conscious rats

J.N. Garcia, N.P. Pedersen, E. Nalivaiko, W.W. Blessing *

Departments of Physiology and Medicine, Centre for Neuroscience, Flinders University, Bedford Park, S.A. 5042, Australia

Received 24 August 2000; received in revised form 23 October 2000; accepted 23 October 2000

Abstract

We describe a surgical procedure for chronically implanting a Doppler ultrasonic probe around the tail artery of the rat to measure phasic flow velocity in the tail artery of the unrestrained conscious rat. The phasic tail flow signal is highly correlated with the simultaneously recorded superior mesenteric flow signal (range 0.70–0.89 in seven rats) during vasoconstriction induced by exposure to formaldehyde vapour. In response to two quick alerting taps on the cage, tail flow velocity fell from 20 ± 2 to 7 ± 1 cm/s ($P < 0.01$) and mesenteric flow fell from 30 ± 5 to 25 ± 4 cm/s ($P < 0.05$), with the fall in tail flow being significantly greater than the fall in mesenteric flow ($P < 0.05$, $n = 7$ rats). In anesthetized rats, the phasic tail flow signal was highly correlated with phasic arterial pressure (range 0.71–0.83 in seven rats). The ability to reliably measure phasic arterial tail flow in the conscious unrestrained rat should facilitate experimental studies of brain pathways regulating flow to this principally cutaneous vascular bed in different physiological situations.

Keywords: Skin blood flow; Cutaneous blood flow; Temperature regulation; Stress; Anxiety

1. Introduction

The principally cutaneous vascular bed in the rat tail is specialized for exchanging heat between the animal and the environment. Thus a number of experimental studies of temperature regulation measure parameters which depend on arterial blood flow to the rat tail (Raman et al., 1983; O'Leary and Johnson, 1989; Nakajima et al., 1998, 1999 and references listed therein). These studies have used tail temperature and tail plethysmography (volume changes) as indices of tail arterial flow in the anesthetized animal and in the restrained conscious animal. Phasic arterial flow has been measured in the tail in anesthetized rats, with a Doppler ultrasonic probe placed just after the origin of the caudal artery from the descending aorta in the pelvis (Nakajima et al., 1999). However there have been no direct measurements of tail artery flow in conscious unrestrained rats, and no measurements from the tail artery itself, principally because the anatomical arrangement of the tail artery has made it difficult to implant a probe without damaging the artery (Nakajima et al., 1999).

Neural regulation of body temperature is significantly altered by anesthesia, so that a tail arterial flow measurement technique applicable to the conscious rat would be valuable. Beat-to-beat measurement of tail artery flow in the conscious unrestrained rat would also enable assessment of cutaneous flow in various natural situations. It would be possible to determine, for example, whether cutaneous blood flow in rats suddenly falls when the animal detects a salient environmental event, as is the case in humans and rabbits (Wallin and Fagius, 1988; Yu and Blessing, 1997a, 1999).

We now report a surgical technique which makes it possible to implant and chronically maintain an ultrasonic Doppler probe around the rat tail artery, so that beat-to-beat arterial blood flow can be continuously monitored either in the anesthetized animal or in the unrestrained conscious animal.

* Corresponding author. Tel.: +61-8-82044736; fax: +61-8-82045450.

E-mail address: w.w.blessing@flinders.edu.au (W.W. Blessing).

0165-0270/01/$ - see front matter
PII: S0165-0270(00)00347-2

2. Methods

Male Sprague Dawley rats (350–450 g) were anesthetized with 1–2% halothane in oxygen. The animal was placed in the supine position. The ventral surfaces of the coccygeal vertebrae were palpated to determine the thinner middle portion of the vertebra, between the prominances at the distal extent in the region of the intervertebral discs. We made a 2 cm longitudinal midline skin incision, on both the ventral and dorsal surfaces, centred over the midportion of one of the vertebrae (fifth to the eighth coccygeal level). The skin on ventral and dorsal sides of the tail was gently dissected from the underlying tendons and fascial sheaths. A 0.5 cm midline longitudinal incision was made through the fascia overlying the ventral tail artery and the vessel was gently isolated from the surrounding tissue and from the underlying veins for $\sim$0.5 cm. A drop of 1% lignocaine was applied to the exposed vessel.

The mobilized portions of the ventral tail artery and the veins were gently moved slightly laterally from their midline position and the underlying bone was exposed. A hole was drilled through the vertebral body from the ventral to the dorsal extent. A piece of 14 gauge steel tubing was passed from the ventral surface through the drilled hole so that it protruded from the skin on the dorsal surface of the tail. The distal ends of the insulated wires of the Doppler probe (Iowa Doppler Products, Iowa, 0.8 mm probe) were fed into the ventral end of the tubing and passed through so that they protruded from the dorsal end. The tubing was then pulled through the vertebra and removed by threading it along the insulated wires on the dorsal side. The insulated wires, protruding through the skin on the dorsal side of the tail, were then grasped by forceps, pulled back through the skin, and passed subcutaneously to the interscapular region.

It also proved feasible to pass the probe wires between the tendons and the lateral side of the vertebra, rather than passing through the bone itself (Fig. 1). The operation was again performed with the rat in the supine position. The ventral tail artery was mobilized and moved laterally as described above. The skin was separated from the underlying tendons on one side of the tail. A fine pair of forceps was then passed under the bunch of tendons, medially towards the vertebral body, commencing just ventral to the lateral tail vein. The points of the forceps were then directed ventrally so they passed between the tendons and the bone and exited in the ventral midline, medial to the laterally displaced artery. The ends of the probe wires were grasped in the forceps and the wires pulled between the tendons and the bone until the probe was snugly positioned in the midline beside the laterally displaced artery. The artery was then placed within the probe and the wires secured in place with cyanoacrylate glue as described below.

After probe implantation by either procedure, a small incision was made in the skin in the interscapular region and a long thin pair of forceps was passed subcutaneously along the back, right down to the tail. The ends of the insulated wires were grasped and pulled subcutaneously so that they exited through the interscapular incision. The neck of the probe was positioned in the space made by the needle puncture, so that the probe itself rested snugly in the midline, beside the laterally displaced artery. The artery was then returned to its midline position and positioned within the Doppler probe. Cyanoacrylate glue was used to fix the insulated wires to the surrounding tissue on the dorsal and the ventrolateral sides of the tail. The dorsal and ventral midline skin incisions were sutured. The wires were secured subcutaneously in the interscapular region, with $\sim$2 cm of each wire left protruding through the skin.

In some rats we placed an additional Doppler probe (diameter 1.6 mm) around the origin of the superior mesenteric artery. The wires were brought out through the abdominal wall and secured subcutaneously beside the tail probe wires in the interscapular area.

Anesthesia was discontinued and the animal placed in its cage and returned to the animal house. After recovery for at least 1 week the animal, in its usual cage, was transferred from the animal house to the laboratory. The top of the cage was removed and the animal was provided with plenty of wood shavings to make a nest. The temperature of the cage was main-

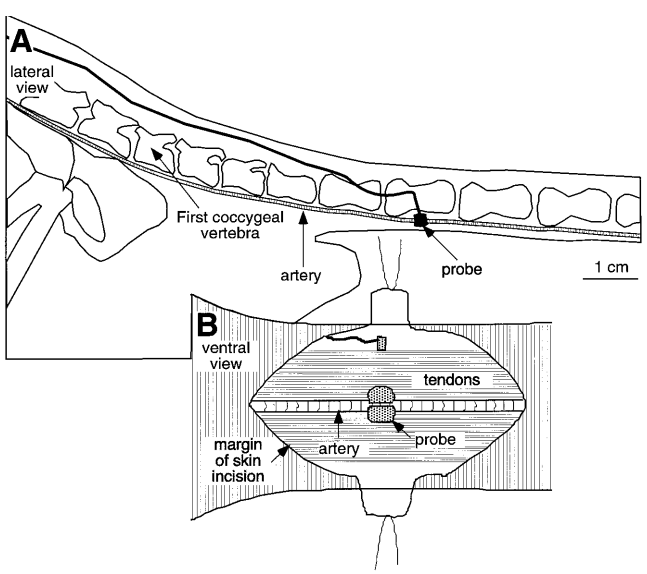

Fig. 1. (A) Diagram of a lateral view of the position of the implanted Doppler probe, based on an X-ray of an anesthetized rat. (B) Diagram of a ventral view of the tail. The skin has been incised and retracted to view the tendons. The probe has been positioned by passing the wires between the tendons and the bone.

tained between 24 and 26°C. A cable suspended above the cage connected the protruding Doppler wires to an analysing system (Triton Technology, San Diego, CA). The resulting analogue Doppler signal was digitized (40 or 100 Hz) with MacLab (ADInstruments, Sydney, Australia) connected to a Macintosh G3 computer programmed with MacLab Chart software. The signals were analysed with Chart and with IgorPro software (Wavemetrics, OR). The ultrasound Doppler signal was calibrated using the internal standards in the Triton system, with values expressed as cm/s.

When tail and mesenteric flow signals were stable, a stream of formaldehyde vapour was directed at the rat's nostrils. The animal was observed to stop breathing. Phasic tail and mesenteric Doppler signals were recorded during the animal's response to this stimulus. After the two flow signals had returned to normal levels and stabilized we tested the response to tapping the outside of the cage with the handle of a pair of forceps. The noise of the taps (two quick sequential taps) was recorded with a microphone and displayed on the record.

Some rats with tail probes chronically implanted were anesthetized by exposure to 1–2% halothane in oxygen and subsequently with urethane (1.5 g/kg infused into the external jugular vein over 30 min). A catheter was placed into the left common carotid artery for measurement of arterial pressure. An endotracheal tube was inserted to protect the airway and for subsequent delivery of 100% oxygen or 1–2% halothane in oxygen. Phasic arterial pressure and phasic tail artery Doppler signals were monitored, and when the arterial pressure was stable a 5 min record sample was selected for each rat.

Tail and mesenteric flows were analysed by two procedures. Mean values for 5 s periods before and 5–10 s after formaldehyde presentation or cage tapping were calculated for each rat in each experimental condition and analysed with repeat measures analysis of variance. Phasic and mean (1s bins) tail and mesenteric flow (conscious rats), and tail flow and arterial pressure (anesthetized rats) were correlated using the Pearson coefficient.

3. Results

Fig. 2(A) shows a sample of the phasic tail artery Doppler signal recorded in a conscious rat 1 week after probe implantation. At the time of recording the animal was unrestrained in its usual cage (24–26°C), resting in a wood-shaving nest. The flow velocity signal has an obvious pulsatile component, with the trace varying, in this animal, between ~ 10 and 28 cm/s. In seven conscious rats in which the tail trace was at a reasonably stable level (see below for discussion of variability of

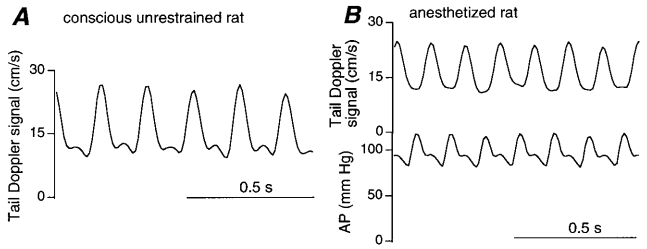

Fig. 2. (A) MacLab records of the ultrasonic Doppler tail artery flow signal from an unrestrained conscious rat, with the Doppler probe implanted around the base of the tail artery 1 week before the recording. (B) Simultaneously recorded flow signal from the tail artery Doppler probe and arterial pressure signal (AP) recorded from a catheter in the common carotid artery; anesthetized rat, Doppler probe implanted 1 week before the recording.

cutaneous flow) the minimum diastolic flow velocity signal was 13.6 ± 11.5 cm/s and the maximum systolic flow velocity signal was 32 ± 23 cm/s (mean $\pm$ SD).

A stream of formaldehyde vapour was directed at the nostrils of the conscious rat. The resulting transient apnoea was accompanied by a marked bradycardia (from 380 ± 12 to 117 ± 7 beats/min, $n = 7$ rats, $P < 0.01$) and marked reductions in both the tail and the mesenteric Doppler flow signals (Fig. 3(A)). In seven rats, tail flow velocity fell from 23 ± 5 to 11 ± 4 cm/s ($P < 0.01$) and mesenteric flow velocity fell from 28 ± 4 to 14 ± 3 cm/s ($P < 0.01$). Formaldehyde had equivalent effects on tail and mesenteric signals ($P > 0.05$). For each of the seven rats we selected from the phasic records a 35 s epoch which included a brief control period and the response to formaldehyde vapour, as shown in Fig. 3(A). The two flow velocity signals were out of phase by ~ 25 ms. The tail signal was advanced by this time and we then calculated Pearson correlations between phasic tail flow and mesenteric flow signals for the seven animals. The correlations were 0.89, 0.74, 0.83, 0.70, 0.73, 0.87 and 0.71. In Fig. 3(A) the tail and mesenteric flow records display an irregularity which reflects a cardiac arrhythmia sometimes seen after administration of formaldehyde. It is clear from the records, and documented by the 0.89 correlation value, that the two different probes detect the arrhythmia-induced flow changes in a remarkably similar manner. When the two flow signals were averaged in 1s bins to eliminate the phasic components, the corresponding correlation coefficients for the seven rats were 0.61, 0.86, 0.96, 0.45, 0.98, 0.85 and 0.69.

The similarity in the tail and mesenteric flow responses to formaldehyde contrasted with the different magnitudes of the response to cage tapping. As seen in Fig. 3(B), two quick taps on the side of the cage caused a much greater fall in the tail flow signal than in mesenteric flow signal. In seven rats, tail flow fell from 20 ± 2 to 7 ± 1 cm/s ($P < 0.01$) and mesenteric flow fell from 30 ± 5 to 25 ± 4 cm/s ($P < 0.05$), with the fall in

tail flow being significantly greater than the fall in mesenteric flow ($P < 0.05$). In conscious rats, tail flow sometimes rapidly decreased to near zero levels, apparently spontaneously or clearly in response to a salient 'naturally occurring' environmental event, for example the ringing of the laboratory telephone. Mesenteric flow was much more stable.

In undisturbed halothane- or urethane-anesthetized rats, resting arterial pressure was usually stable over minutes. We examined the relationship between the phasic arterial pressure signal and the phasic tail flow signal during a 5 min period in each of seven rats. The two signals were out of phase by ~ 50 ms. When the tail flow signal was advanced by this time interval the Pearson correlation coefficients between phasic arterial pressure and tail flow signals were 0.78, 0.75, 0.83, 0.83, 0.82, 0.71 and 0.71. A sample of pressure and flow records from an anesthetized rat is shown in Fig. 2(B).

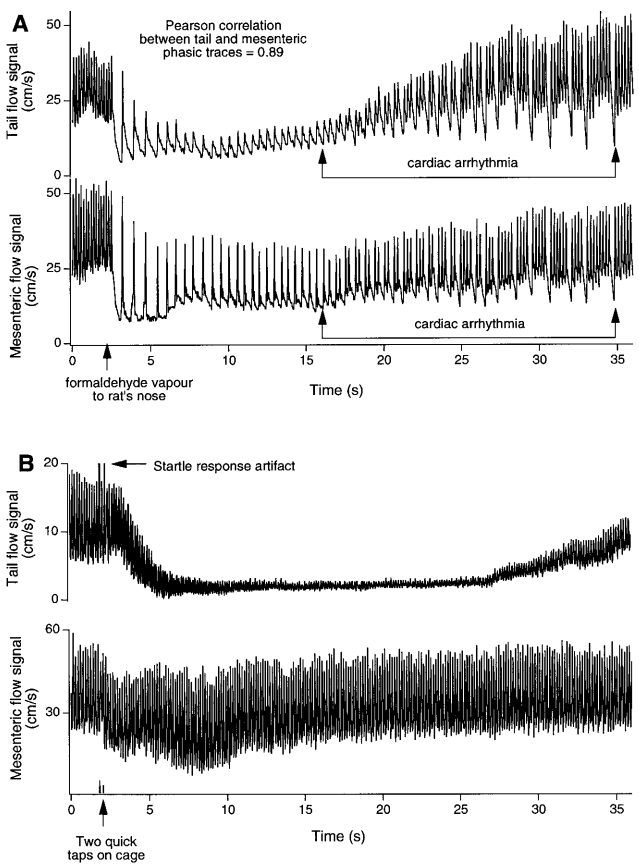

Fig. 3. (A) MacLab records of phasic ultrasonic Doppler signals recorded simultaneously from tail and superior mesenteric arteries. Formaldehyde vapour was directed towards the rat's nostrils at the time indicated by the baseline arrow. During the recovery phase, a transient cardiac arrhythmia affects the flow in both probes. (B) MacLab records of ultrasonic Doppler signals recorded simultaneously from tail and superior mesenteric arteries. The rat's cage was quickly tapped twice at the time indicated by the baseline arrow.

4. Discussion

We believe that our study is the first to record phasic arterial blood flow to the tail, a principally cutaneous vascular bed, in the unrestrained conscious rat.

The surgical implantation procedure is relatively simple. As long as the artery is dissected gently, with minimal trauma, and placed within a probe resting in the midline snugly against the bone, robust Doppler flow signals are usually obtained from the time of probe implantation. The muscles which move the tail are principally proximal to the level chosen for probe implantation (Hebel and Stromberg, 1976), so that the arterial flow that we measured is distributed principally to the cutaneous bed.

The chronically implanted Doppler ultrasonic probe system used in our study has been well validated in a number of vascular beds (Haywood et al., 1981). The probes provide robust signals proportional to average arterial red cell velocity in the artery being studied. The valid inference from velocity to flow requires knowledge of the cross sectional area of the artery in the region of the probe. As long as this area remains constant, changes in the flow are directly proportional to changes in the velocity signal, so that in a single animal relative changes in flow can be accurately assessed.

In the anesthetized rat, we assessed the reliability and validity of the tail flow signal by comparing it with the phasic arterial pressure signal at a time when the animal was in a resting, unstimulated state. The correlation between the two signals was very high. In the conscious rat, we assessed the reliability of the tail flow phasic Doppler signal by comparing it with the simultaneously recorded superior mesenteric phasic Doppler flow signal at rest and in response to formaldehyde vapour and to tapping of the cage. Formaldehyde vapour reduced tail flow in the same manner in which it affected mesenteric flow. The correlation coefficients between the two signals were very high, attesting to the reliability of the tail recordings. Bradycardia and increase in arterial pressure in response to smoke inhalation has previously been reported in conscious rats (Nakamura and Hayashida, 1992; Houdi et al., 1995), but as far as we are aware the present study is the first report of changes in mesenteric and cutaneous beds in response to inhalation of noxious vapour. The cutaneous response in the rat is similar to the corresponding response in the rabbit (Yu and Blessing, 1997b).

In the conscious rat, tapping the cage, an alerting stimulus, reduced tail flow much more than it reduced mesenteric flow. Since the rat tail is a principally cutaneous vascular bed, our study suggests that rats are generally similar to rabbits (Yu and Blessing, 1997a, 1999) and humans in the manner in which cutaneous blood flow is selectively sensitive to salient environmental events (see references in Wallin and Fagius, 1988).

In rabbits, sudden alerting-related falls in ear pinna flow are preceded by a sudden increase in the proportion of theta rhythm in the hippocampal EEG, an indication that the animal has detected a salient, possibly threatening stimulus (Yu and Blessing, 1997a). These falls in cutaneous flow are prevented by inactivation of neuronal function in the region of the amygdala (Yu and Blessing, 1999). This tendency of cutaneous vessels to constrict in animals subjected to alerting stimuli emphasizes the importance of ensuring that the rat is unstressed when arterial pressure is being measured by the tail cuff method in the unanesthetized restrained rat (Pfeffer et al., 1971; Widdop and Li, 1997).

Measurement of rat tail blood flow by the methods outlined in our paper should facilitate experimental studies of the neural regulation of the cutaneous circulation in the conscious animal, including studies of body temperature regulation. The surgical procedure for probe implantation, although straight-forward, does cause local trauma at the time of implantation, possibly temporarily changing the responsiveness of the tail vessel. After the normal healing process, a chronically implanted probe can measure tail flow with minimal interference with the animal's normal physiological regulation of the tail vascular bed. Use of chronically implanted probes should thus also facilitate studies of brain and spinal regulation of the cutaneous circulation in anesthetized rats.

Acknowledgements

Our research was supported by the National Health and Medical Research Council, by the National Heart Foundation of Australia and by the Neurosurgical Research Foundation of South Australia. Kate Barber and Robyn Flook provided technical assistance.

References

Haywood JR, Shaffer RA, Fastenow CF, Fink GD, Brody MJ. Regional blood flow measurement with pulsed Doppler flow meter in conscious rat. Am J Physiol 1981;241:R273–8.

Hebel R, Stromberg MW. Anatomy of the Laboratory Rat. Baltimore: Williams and Wilkins, 1976.

Houdi AA, Dowell RT, Diana JN. Cardiovascular responses to cigarette smoke exposure in restrained conscious rats. J Pharmacol Exp Ther 1995;275:646–53.

Nakajima Y, Nose H, Takamata A. Plasma hyperosmolality and arterial pressure regulation during heating in dehydrated and awake rats. Am J Physiol 1998;275:R1703–11.

Nakajima Y, Nose H, Takamata A. Comparison between tail skin blood flow measurements by ultrasonic Doppler flowmetry and plethysmography during heating in anesthetized rats. Jpn J Physiol 1999;49:121–4.

Nakamura T, Hayashida Y. Autonomic cardiovascular responses to smoke exposure in conscious rats. Am J Physiol 1992;262:R738–45.

O'Leary DS, Johnson JM. Baroreflex control of the rat tail circulation in normothermia and hypothermia. J Appl Physiol 1989;66:1234–41.

Pfeffer JM, Pfeffer MA, Frohlich ED. Validity of an indirect tail-cuff method for determining systolic arterial pressure in unanesthetized normotensive and spontaneously hypertensive rats. J Lab Clin Med 1971;78:957–62.

Raman ER, Roberts MF, Vanhuyse VJ. Body temperature control of rat tail blood flow. Am J Physiol 1983;245:R426–32.

Wallin BG, Fagius J. Peripheral sympathetic neural activity in conscious humans. Ann Rev Physiol 1988;50:565–76.

Widdop RE, Li XC. A simple versatile method for measuring tail cuff systolic blood pressure in conscious rats. Clin Sci 1997;93:191–4.

Yu YH, Blessing WW. Cutaneous vasoconstriction in conscious rabbits during alerting responses detected by hippocampal theta-rhythm. Am J Physiol 1997a;272:R208–16.

Yu YH, Blessing WW. Cerebral blood flow in rabbits during the nasopharyngeal reflex elicited by inhalation of noxious vapor. J Auton Nerv Syst 1997b;66:149–53.

Yu YH, Blessing WW. Amygdala coordinates sudden falls in ear pinna flow in response to unconditioned salient stimuli in conscious rabbits. Neurosci 1999;93:135–41.