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Compensatory mechanisms to maintain blood pressure in paraplegic rats: Implication of central tachykinin NK-1 and NK-3 receptors?

Frank Cloutier [a,b,*], Jenny L. Lauschke [a], Pascal Carrive [a]

[a] Neural Injury Research Unit, School of Anatomy, University New South Wales Sydney, Australia
[b] Research Center, Hôpital Sainte-Justine, Department of Pediatrics, University of Montreal, 3175 Côte Sainte-Catherine, Montreal, Quebec, Canada H3T 1C5

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ABSTRACT

People with high level spinal cord injury (SCI) suffer from both hypotension and spontaneous hypertension due to loss of supraspinal control of spinal sympathetic outflow. Few reports have addressed whether any changes occur in central regulation of blood pressure (BP) and heart rat (HR) at the supraspinal level. Central tachykinin NK-1 and NK-3 receptors are located in many cardiovascular areas in the brain and are known to modulate BP and HR. This study examined the intracerebroventricular (i.c.v.) effects of the selective NK-1 receptor agonist [Sar^9, $Met(O_2)^{11}$]SP (65 pmol, $n = 6$) and NK-3 receptor agonist senktide (650 pmol, $n = 6$) on mean arterial pressure (MAP) and HR before and after complete spinal cord transection at thoracic level 4 (T4). [Sar^9, $Met(O_2)^{11}$]SP evoked increases in MAP and HR which were still present 4 days after the T4 SCI. Further analysis using the β_1-adrenoceptor antagonist atenolol (10 mg kg^{-1}) revealed an increased contribution of HR in the MAP increase after SCI. For senktide, 2 and 5 weeks after T4 SCI, the rise in MAP induced by senktide was significantly increased in magnitude and was similar to a normal response at 8 weeks. These effects were accompanied by a bradycardia, which was still present and amplified at 8 weeks. Our results reveal a transient potentiation of the senktide-mediated MAP effect and a greater contribution of the HR in MAP increase by [Sar^9, $Met(O_2)^{11}$]SP in T4 transected rats. Although the significance of these changes remains to be established. This suggest a reorganization of supraspinal mechanisms regulating BP and HR after a high level SCI. Central NK-1 and NK-3 receptors might therefore contribute to the maintenance of MAP following high thoracic SCI.

1. Introduction

It is well known that spinal cord injury (SCI) is a devastating event that leads to permanent motor and sensory impairments caudal to the level of injury. In addition, autonomic disturbances, in particular cardiovascular dysregulation, may add to the lifelong impairment. Data published in recent years has revealed that cardiovascular diseases in the acute and chronic stages of SCI are now among the most common causes of death in individuals with cervical and upper thoracic SCI (DeVivo et al., 1999; Frankel et al., 1998; Hartkopp et al., 1997).

Cardiovascular abnormalities are attributable to the loss of supraspinal control on spinal sympathetic outflow with the level of injury being critical. SCI above the level of the sixth thoracic segment (T6) results in significantly reduced sympathetic outflow as well as loss of supraspinal control to both the splanchnic and lower limb vascular bed. The consequences are immediate. Patients experience a period of "spinal shock" characterized by a profound drop in BP, particularly with cervical injury (Mathias and Francel, 1992). In such conditions, vasopressive therapy is required to maintain normal blood pressure (Bilello et al., 2003; Furlan et al., 2003; Glenn, 1997; Krassioukov and Claydon, 2006; Mathias et al., 1979; Vale et al., 1997).

As we and others have reported, the same hypotension can be obtained in a rat model of complete spinal cord transection at T4 (Choi et al., 2005; Krassioukov and Weaver, 1995; Laird et al., 2006, 2008; Maiorov et al., 1997). MAP initially falls for several days following SCI and then stabilizes, but remains approximately 20 mm Hg lower than prior to injury for at least up to 6 weeks (Laird et al., 2006). The concomitant increase in HR and the partial regain of MAP levels suggest that compensatory mechanisms operate after the SCI.

One report has revealed that such mechanisms can originate from the supraspinal level. Vasopressin (VP) release is modulated by central cholinergic receptors and intracerebroventricular

Abbreviations: aCSF, artificial cerebrospinal fluid; HR, heart rate; i.c.v., intracerebroventricular; MAP, mean arterial blood pressure; SCI, spinal cord injury.

* Corresponding author. Address: Research Center, Hôpital Sainte-Justine, Department of Pediatrics, University of Montreal, 3175 Côte Sainte-Catherine, Montreal, Quebec, Canada H3T 1C5. Tel.: +1 514 657 3587.
E-mail address: frank.cloutier@hotmail.com (F. Cloutier).

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(i.c.v.) injection of choline in rats produces increases in MAP and plasma VP level that are greater after a complete cervical transection compared to sham operated rats (Savci and Ulus, 1998). This raises the possibility that other neuromodulators of MAP and HR might also be involved.

Tachykinin NK-1 and NK-3 receptors play an important role in the central regulation of MAP and HR (Couture et al., 1995; Culman et al., 1997; Culman and Unger, 1992; Itoi et al., 1992; Takano et al., 1990; Tschope et al., 1992; Unger et al., 1981). I.c.v. injection of natural or selective NK-1 receptor agonists in the conscious freely moving rat leads to increase in MAP, HR and cardiac output (Itoi et al., 1992; Tschope et al., 1992; Unger et al., 1988). On the other hand, i.c.v. administration of NK-3-selective agonist induces increases in MAP (Cellier et al., 1997; Polidori et al., 1989a,b). Although the tachykinins have been reported to act mainly on hypothalamic neurones (Itoi et al., 1991; Massi et al., 1991), the cardiovascular responses induced by NK-1 receptor agonists have been associated with an increased sympathoadrenal activity (Unger et al., 1981; Takano et al., 1990), while those induced by i.c.v. injection of NK-3 agonists result mainly from the release of VP from the hypothalamus and to a minor extent by activation of the sympathetic nervous system (Polidori et al., 1989b; Takano et al., 1990, 1993).

In addition, the cardiovascular effects induced by i.c.v. tachykinins are accompanied by a specific behavioural arousal reaction. The prevailing responses to activation of NK-1 receptor is grooming and face washing, while activation of the NK-3 receptor induces mainly wet dog shake behaviour (Cellier et al., 1997; Itoi et al., 1992; Picard et al., 1994; Tschope et al., 1992; Unger et al., 1988). This reflects a role as a mediator of stress response in the brain (Culman et al., 1995a,b, 1997).

This study aims at determining whether the cardiovascular and the behavioural responses induced by the activation of NK-1 and NK-3 receptors are modified after a complete SCI at T4 in rats. This was achieved by measuring the MAP, HR and behavioural responses elicited by i.c.v. injection of the selective NK-1 receptor agonist [Sar^9, $Met(O_2)^{11}$]SP and NK-3 receptor agonist senktide before and after a complete spinal cord transection at thoracic level 4 (T4).

2. Materials and methods

2.1. Animal source and care

Experiments were performed on 12 adult male AAW rats (350–400 g) obtained from the Biological Resource Center, Sydney, New South Wales. They were housed in individual plastic home boxes (65 × 40 × 22 cm) with *ad libitum* food and water and maintained on a 12/12 h light/dark cycle. All experimental procedures were approved by the Animal Care and Ethics Committee of the University of New South Wales in accordance with guidelines of the National Health and Medical Research Council of Australia for animal research.

2.2. Implantation of permanent i.c.v. cannula

The animal's head was secured in a stereotaxic apparatus and a midline incision was made on the scalp. The angle of the head was adjusted to the horizontal plane with respect to both *bregma* and *lambda* reference points. Four holes were drilled in the bone; three for skull screws and one for the guide cannula (26 G, Plastics One Inc., Roanoke, VA, USA). The guide cannula was implanted into the right lateral ventricle according the stereotaxic coordinates of previous studies (Cloutier et al., 2004). The guide cannula was anchored to the skull and the screws with dental cement. Animals received the antibiotic cephalothin (10 mg kg^{-1} s.c.) and the analgesic carprofen (50 mg kg^{-1} s.c.) daily for 2 days. The correct position of the i.c.v. guide cannula was verified by post-mortem examination at the end of experiment.

2.3. Radio-telemetric probe implantation

One week after the i.c.v. cannula implantation, rats were chronically implanted with radio-telemetric probes (PA-C40, Data Sciences International St. Paul, MN, USA.) for the measurement of MAP and HR. The probes were prepared for implantation by sterilization with a 2% glutaraldehyde solution for 24 h prior to use, as well as a final rinse in sterile saline solution for 30 min prior to implantation. Under anaesthesia with a combination of ketamine and xylazine as above, the ventral abdomen and the inner thigh region on the side for the radio-telemetric probe placement was shaved and scrubbed with an antiseptic solution. The skin was opened over the femoral vessels and, using blunt dissection, a subcutaneous pocket was formed between the caudal edge of the ribcage and the most cranial extension of the knee's range of motion. The radio-telemetric probe was placed in the pocket and secured by passing a 5-0 suture through the surrounding tissues. The probe catheter was inserted into the right femoral artery and the tip advanced until the abdominal aorta above the iliac bifurcation. Antibiotic (cephalothin, 30 mg kg^{-1} s.c.) was administered postoperatively. Post-operative observations were made until full recovery of each animal, to assess poor hindlimb vascular perfusion (observed as a cyanotic hindpaw). Previous studies have shown that there are enough collateral circulations, including the internal iliac artery that connects to the popliteal artery posterior to the knee, to circumvent the femoral artery occlusion (Herzog et al., 2002; Prior et al., 2004; Westerweel et al., 2005). In the present study, no rats developed hind limb distress.

2.4. Spinal cord transection

Two weeks after the radio-telemetric probe implantation, rats were anaesthetised with a combination of ketamine and xylazine as above. The dorsal region between the neck and hind limb, extending approximately 2 cm bilaterally from the spine, was shaved and the clipped area was scrubbed with an antiseptic solution. The rat was placed on a sterile drape with a homeothermic blanket control unit (Harvard apparatus Ltd., Kent, UK) and rectal temperature was maintained at 37 ± 1 °C by the blanket control unit. Bupivacaine (0.5%) was used locally at the incision site and on the spinal cord during surgery. Following dissection of the superficial and deep muscles of the back, a laminectomy of the T3 vertebra was performed to reveal the T4 spinal segment. The dura was cut longitudinally and laterally at the most proximal and distal borders of the laminectomy. After immobilization of the spinal process rostral to the laminectomy site, the spinal cord was then completely transected with microscissors. Complete transection was confirmed with a scalpel blade and observation under the dissecting microscope. Following transection, a gap of approximately 1–2 mm was present between the rostral and caudal ends of the cord and a piece of absorbable gelatin sponge (SPONGOSTAN®, Johnson and Johnson Medical Ltd., Sipton, UK) was inserted to fill the gap and reduce bleeding. Another piece of absorbable gelatin sponge was also placed on the dorsal surface of the cord and the muscle tissue and skin were sutured in layers.

2.5. Post-operative care after spinal cord transection

Immediately after surgery, the animals received the muscarinic acetylcholine receptor antagonist atropine methyl nitrate (3 mg kg^{-1}