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Effect of intrathecal pretreatment with taurine on neurological outcome after transient spinal cord ischemia in the rat

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Spinal cord ischemia and the resulting irreversible loss of neurological function is a devastating complication associated with transient aortic occlusion [1,2]. Several experimental studies indicate involvement of multifactorial changes comparable to those in supraspinal structures. This may involve excessive release of excitatory amino acids such as glutamate from spinal parenchyma and resulting activation of *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors [3–5]. Increased intracellular Ca^{2+} influx resulting from the activation of the NMDA receptor calcium ionophore is then believed to activate several classes of intracellular enzyme systems (lipases, endonucleases, and proteases) leading subsequently to irreversible neuronal degeneration [6,7].

We have observed a comparably significant increase in taurine in the spinal dorsal horn after transient aortic occlusion in the rat [3], and an effect of transient brain ischemia on secondary extracellular taurine release has been also reported [8,9]. Although the precise role of taurine in the modulation of neuronal transmission is not known, it has been reported that taurine has the ability to inhibit NMDA receptor-induced calcium influx [10].

The above data would jointly predict that the exogenous activation of these neuromodulatory systems should provide protection under pathological conditions associated with excessive and/or prolonged activation of NMDA receptors. Accordingly, it has been reported that pretreatment of hippocampal slices with taurine (2 mM) provides significant protection against transient hypoxic episodes, as measured by the recovery of field evoked potentials [11]. To our knowledge, at present, there are no experimental data on the effect of taurine treatment on the recovery of function in models of transient spinal or brain ischemia in vivo.

Based on the above commentary, in the present study, by using a well-defined in vivo model of spinal cord ischemia in the rat [12], we thought to characterize the effect of *intrathecally* administered taurine on the recovery of function and histopathology after an injurious interval of spinal cord ischemia.

The experiments were carried out according to protocols approved by the Institutional Animal Care Committee of the University of California, San Diego. Under halothane anesthesia (2%–2.5%) male Sprague-Dawley rats (300–400 g) were implanted with lumbar intrathecal catheters [13]. Five days after implantation, all animals were anesthetized (1.5% halothane) and prepared for the induction of reversible spinal cord ischemia by previously described techniques [12]. Briefly, following induction, a 2 Fr Fogarty catheter was placed into the descending thoracic aorta through the left femoral artery. To control the proximal arterial blood pressure at 40 mmHg during the period of aortic occlusion, the left carotid artery was cannulated with a 22G teflon catheter and the blood was allowed to flow into an external reservoir during the period of aortic occlusion. To induce spinal cord ischemia, the balloon was inflated with 0.05 ml of saline following injection with 200 units of heparin into the tail artery. At 10 min after the induction of transient spinal cord ischemia, the balloon was deflated and the blood was reinfused for 60 s. All catheters were then removed, the wounds were sutured, and the animals were allowed to recover.

The treatment protocol was divided into two separate studies: (A) assessment of the effect of increasing doses of intrathecally administered taurine in control

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nonischemic animals, and (B) assessment of the effect of intrathecal pretreatment with taurine on the outcome after an injurious (10 min) interval of spinal ischemia.

To investigate the effect of intrathecal taurine on the cardiopulmonary system and neurological function, in study A halothane (1.5%)-anesthetized rats were injected intrathecally with sequentially increasing doses of taurine dissolved in 10 µl of saline as follows: 1.2 nmol ($n = 5$), 12 nmol ($n = 5$), 60 nmol ($n = 5$), 120 nmol ($n = 4$), and 480 nmol ($n = 4$). After recovery from halothane anesthesia, the effect of the above treatment on the motor (walking function, righting reflex) and sensory function (light touch, pinching) was assessed for a period of 24 h after injection. Based on the data from this study, three doses were then selected to study the effect of such treatment on the recovery of function after transient spinal ischemia (study B).

In study B, animals were prepared for the induction of spinal ischemia. Ten minutes before aortic occlusion, 1.2 nmol ($n = 5$), 12 nmol ($n = 5$), or 60 nmol ($n = 5$) of taurine was injected intrathecally. In the control group, the rats ($n = 4$) were given a total of 10 µl of 0.9% saline. Spinal cord ischemia was then induced for 10 min. After ischemia, animals were allowed to recover and survived for 2 days. During this period the recovery of neurological (motor and sensory) function was assessed using the following criteria: Motor function: (a) normal (full recovery of motor function), (b) knuckle walking (unable to stand but able to knuckle walk), (c) paraplegia (presence of spastic or flaccid paraplegia with complete loss of knuckle walking). Sensory function: (a) nonresponsiveness (absence of any motor or vocal response to pinching of the right hind paw), (b) exaggerated sensory response (evocation of vigorous squeaking and agitation in the response to light stroking of the flank).

After the animals had been perfused transcardinally with 150 ml of 4% paraformaldehyde in phosphate buffer at the end of the survival period, the spinal cords were removed, followed by dissection of L_3–L_5 spinal segments, which were subsequently prepared at 1 µm in thickness and stained with p-phenylenediamine.

In study A, intrathecal delivery of taurine in control halothane-anesthetized animals at doses above 120 nmol evoked respiratory arrest, detected by observing the movements of the thoracic wall during the initial 2–5 min after injection (Table 1). Animals that received taurine at doses less than 60 nmol did not display motor or sensory dysfunction.

In study B, in the control saline-injected group, all animals displayed acute spastic paraplegia for the initial 24 h of reperfusion, and the majority (3/4) of animals remained spastic or flaccid for an additional 24 h of survival (Fig. 1). At 48 h of survival, two of four animals showed complete loss of sensory function, while two retained some responsivity to a pinch of the lower extremities (Fig. 1). Intrathecal pretreatment with taurine at all doses tested had no significant effect on the recovery of motor or sensory function, and all animals showed a comparable degree of neurological dysfunction to that seen in saline treated animals.

Consistently with the presence of severe motor dysfunction, histopathological analysis of the lumbar transverse spinal cord sections revealed the presence of extensive gray matter necrosis localized between laminae III to VII (Fig. 2). No differences between saline- and taurine-treated animals were seen.

In the present study, no protective effect after intrathecal pretreatment with taurine was seen, as assessed by both the recovery of neurological function and the extent of histopathological changes. Although the lack of protection may simply reflect the difference between in vitro and in vivo preparations, several methodological issues should also be considered.

Dose of intrathecally injected taurine. In the present study, in halothane-anesthetized animals, intrathecal taurine administration at doses above 120 nmol caused respiratory arrest 2–5 min after injection. Thus, the maximum dose employed in the ischemia-recovery study (60 nmol) was the highest dose that was possible to use without significant respiratory depression. However, it is also important to note that in the preliminary

Table 1. Side effects of intrathecally administered taurine

Dose (nmol)	Cardiopulmonary	Motor function	Sensory function
1.2 ($n = 5$)	N.S.[a]	N.S.	N.S.
12 ($n = 5$)	N.S.	N.S.	N.S.
60 ($n = 5$)	N.S.	N.S.	N.S.
120 ($n = 4$)	Resp. arrest	—	—
480 ($n = 4$)	Resp. arrest	—	—

[a] No significant detectable effects.

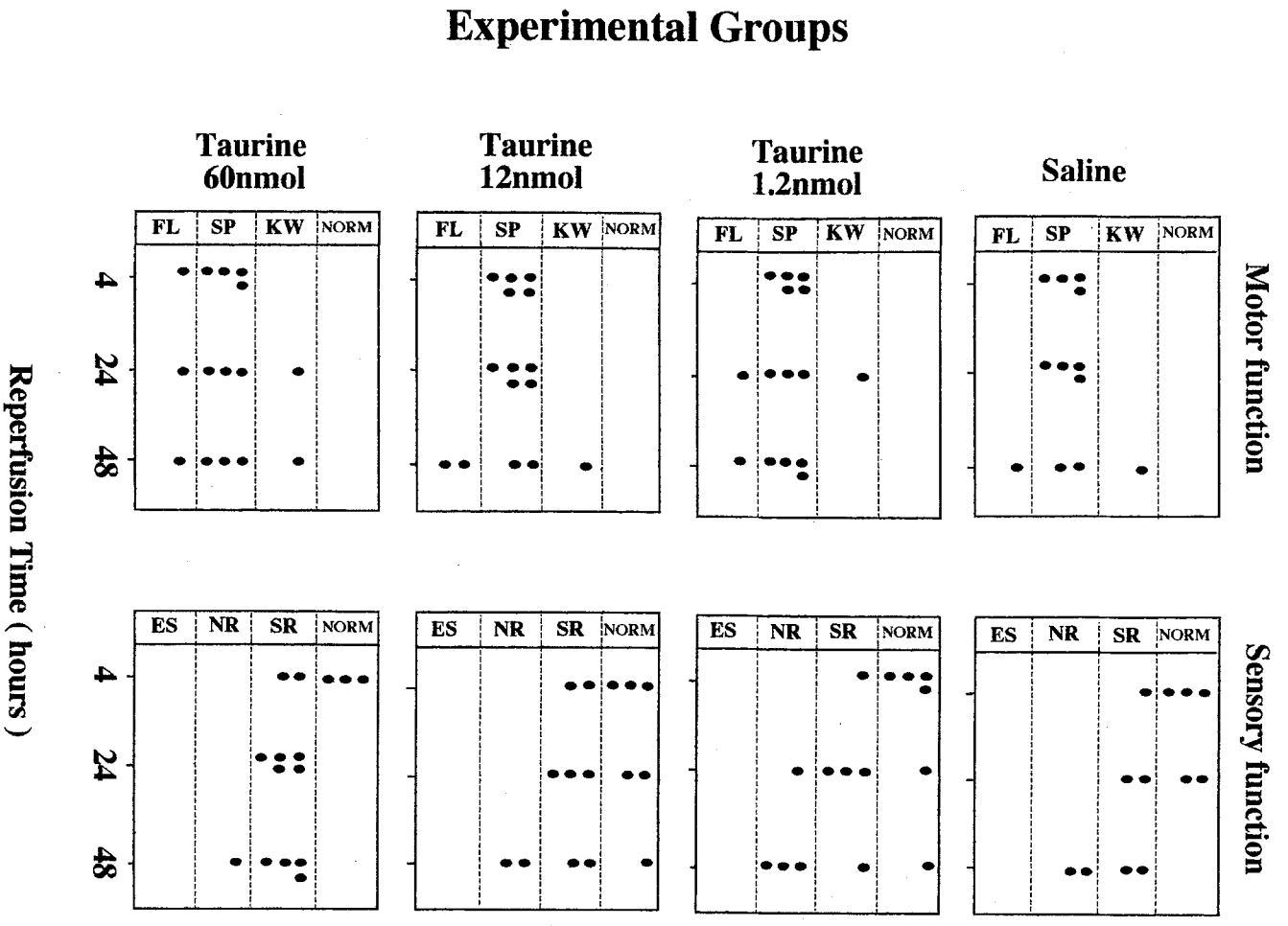

Fig. 1. Motor and sensory states assessed at 2, 24, and 48 h after 10 min of aortic occlusion in groups of rats pretreated intrathecally with taurine at 1.2, 12, and 60 nmol. NORM indicates normal motor or sensory function; KW, knuckle-walking; SP, spasticity; FL, flaccidity; SR, some response; NR, no response; ES, exaggerated sensory

study, in awake animals administration of taurine at doses of 5100 nmol had no detectable side effects, and all animals survived without noticeable deficits. In a microdialysis study [3] using the spinal ischemic model, the concentration of taurine in spinal cord was reported to increase to 2~4 mM during reperfusion from 0.8~1.2 mM in the preischemic condition. These data presume that intrathecal injection with 60 nmol per 10 µl (6 mM) of taurine in the current study would raise the concentration of taurine in the spinal cord to the same level as during reperfusion, and would be also an adequate dose in CSF compared with the in vitro study (taurine: 2 mM) [11].

Clearance and metabolism of taurine in spinal CSF. One explanation for the lack of the protective effect of intrathecal taurine treatment may be associated with rapid metabolism and/or clearance of taurine from spinal CSF. However, it has been reported that about 80% of [^{3}H]taurine injected into the cerebral ventricle remains in the CNS tissue 90 min after the injection in the rat [14]. We therefore assume that the majority of the injected taurine in the present study retained sufficient activity several hours after intrathecal administration and that insufficient availability of taurine in the spinal extracellular space will not likely be involved in lack of the protective effect.

Severity of ischemic insult. In the present study, a 10 min ischemic interval was used to test the possible protective effect of intrathecal pretreatment with taurine. This interval was chosen on the basis of our previous experiments, which showed that in this model, 8 min of spinal ischemia had minimal or no deleterious effect on spinal neuronal function, with nearly complete recovery observed after 24–48 h of reperfusion. However, 10 min of spinal ischemia evokes spastic or flaccid paraplegia in the majority of animals [12]. Importantly, in recent studies employing the same ischemic model, we found that intrathecal pretreatment with noncom-

Fig. 2. Histopathological analysis of spinal cord (L_4 level) from rat pretreated with 60 nmol of taurine at 48 h survival after aortic occlusion. Complete spastic type of paraplegia. Extensive necrosis affecting central part of the gray matter and some α-motor neurons can be seen in the ventral horn

petitive NMDA antagonists provided significant protection after 10 min of spinal ischemia (Taira and Marsala, unpublished observations). These data argue against the notion of nonspecific neuronal degeneration resulting from too severe ischemic insult.

In conclusion, this study showed that intrathecal pretreatment with taurine at doses lower than 60 nmol had no significant effect on either the neurological outcome or the histopathological changes following transient spinal cord ischemia, and at doses higher than 120 nmol caused respiratory depression in halothane-anesthetized rats. Further studies employing other routes of administration with higher doses of taurine without any respiratory depression, for example, intravenously or intraperitoneally, should be performed.

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