

Noradrenaline's neurotoxicity in spinal cord injury and spinal anaesthesia and its role in dental pulp homeostasis

Thesis booklet

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INTRODUCTION

Noradrenaline plays an important role in homeostasis as a neurotransmitter of the central and autonomic nervous system and a hormone. Its primary function is to promote alertness and vigilance, and to control the body's response to stress and danger.

However, despite its physiological importance, noradrenaline can also mediate neurotoxic effects, meaning that the excessive release of the transmitter can damage surrounding neural tissue. Such excessive release can be triggered, as our study shows, by the injury or local anaesthesia of the spinal cord.

The global incidence rate of spinal cord injury is around 180 000 and more than half of this is due to road traffic accidents. Most of the victims are below 30 years of age and become permanently impaired, as current therapeutic procedures cannot achieve significant improvement in neural function.

Apart from the limited regenerative potential of neural tissue, the damage to the spinal cord is worsened by several factors of secondary spinal cord injury, like ischemia, inflammation and excitotoxicity due to the excessive release of neurotransmitters. All three are in close interaction with the release of noradrenaline, thus the transmitter could play an important role in the pathology of spinal cord injury.

Noradrenaline might be equally important in the mechanism of local anaesthesia. Previous studies on the spinal cord proved that the Na^+ and K^+ channel blocker lidocaine significantly increases the release of noradrenaline, which might be due to its K^+ channel blocking effect.

This could promote its anaesthetic and vasoconstrictive properties, but also its neurotoxic effects.

As the neurotransmitter of the sympathetic nervous system and a hormone, noradrenaline is an important regulator of the dental pulp's homeostasis. Noradrenaline is not only the primary mediator of vasoconstriction in the pulp, but also influences its pain perception and inflammatory processes. Some evidence suggests that noradrenaline modulates the size of periapical lesions, the formation of tertiary dentine and the extent of root resorption during orthodontic tooth movement.

OBJECTIVES

- 1) Following an artificial spinal cord injury, we aimed to measure the changes in local noradrenaline release using an ex vivo microperfusion method, at 1, 3 and 14 days after the injury.
- 2) To study the function of the noradrenaline uptake transporter following spinal cord injury, as the transporter is important in non-synaptic noradrenaline release during pathological conditions.
- 3) To determine the effect of the Na⁺ and K⁺ channel blocker local anaesthetic lidocaine on the release of noradrenaline from spinal cord slices, and to compare it with the effect of the selective Na⁺ channel blocker tetrodotoxin and the selective K⁺ channel blocker 4-aminopyridin.
- 4) To test a human dental pulp experimental model that could be used to determine the changes in neurotransmitter release during certain pharmacological and pathological conditions.

METHODS

Tissue samples

Spinal cord slices used in our experiments were isolated from adult female Wistar rats. In the spinal cord injury model, we used 16 animals for the uptake experiments, 49 animals for the fractional release experiments and 2 animals for electron microscopy. For the local anaesthetic model, we used another 12 animals.

Human dental pulp samples were isolated from healthy wisdom teeth from patients less than 25 years of age.

Spinal cord injury

Injury of the spinal cord was simulated with an artificial unilateral cutting (hemisection) at the 4th lumbar segment. Rats were anaesthetised and the spinal cord was exposed using laminectomy, then cut unilaterally with a crescent shaped blade. 1, 3 and 14 days later, rats were decapitated and the spinal cord tissue, 2-5 mm caudally to the injury, removed and cut into 400 μm thick slices. 4 slices were used for each experiment, with an average total weight of 13.1 ± 2.9 mg.

[³H]noradrenaline labeling

Spinal cord slices were preincubated in aerated (95% O₂ and 5% CO₂) Krebs solution for 20 min at 37 °C. In half of the uptake experiments, 1 μM nisoxetine was added to the preincubation and incubation solutions, which inhibits the noradrenaline uptake transporter. Subsequently, 5 $\mu\text{Ci/ml}$ radioactive tritium-noradrenaline ([³H]NA) was added to the solution for another 45 min of incubation. The radioactively labeled transmitter is taken up specifically into noradrenergic terminals due to

the noradrenaline transporter. Tissue slices were then placed into microperfusion chambers. The chambers were preperfused with aerated Krebs solution at 37 °C at a rate of 0.5 ml/min for 60 min.

Measurement of noradrenaline uptake

In the uptake experiments, after preperfusion, tissue slices were homogenised in trichloroacetic acid and their radioactivity was determined with a liquid scintillation spectrometer and expressed in Bq/g (disintegrations/sec/gram of spinal cord tissue).

Stimulated noradrenaline release

In the fractional release experiments, after preperfusion, chamber effluents were collected in 19 consecutive fractions, 200 sec each. Electrical field stimulation (40 V, 3 Hz, 1 msec) was applied with platinum electrodes for 80 sec at the beginning of the 3rd and 13th fractions. In half of the experiments, 1 µM nisoxetine was added to the perfusion solution starting at the 8th fraction. Following fraction collection, we determined the radioactivity of the fractions, which is proportionate to the amount of released [³H]NA. The transmitter release observed in the 4 fractions following electrical stimulation in addition to the resting release was considered stimulated release

Electron microscopy

Transmission electron microscopy images were obtained from healthy samples and 3 days after injury, at the ventral horn of spinal cord segment L5, 2 mm caudally to the injury.

Local anaesthetics

The measurement methods used with local anaesthetics were similar to those used after spinal cord injury. The Na⁺ and K⁺ channel blocker lidocaine was applied at a concentration of 5 mM, which can be achieved clinically during spinal anaesthesia. The concentration of the selective Na⁺ channel blocker tetrodotoxin was 1 μM, and the concentration of the selective K⁺ channel blocker 4-aminopyridin was 300 μM.

Dental pulp experiments

Measurement methods used on human dental pulp were also similar to those described above. Microperfusion chamber effluents were collected in 32 consecutive fractions, 300 sec each. Electrical field stimulation (40 V, 5 Hz, 1 msec) was applied for 30 sec at the beginning of the 7th fraction, and for 100 sec at the beginning of the 17th and 27th fractions.

RESULTS

Morphological changes after spinal cord injury

After surgical hemisection, the rats showed typical signs of hemiplegia, with no movement of the right hindlimb. 1 and 3 days after injury, the macroscopic appearance of the spinal cord resembled healthy tissue. Electron microscopy images revealed an overall degeneration but many intact synapses were observed.

[³H]noradrenaline uptake

Following tissue labeling, the average uptake of [³H]NA in the spinal cord slices from the control group was 149 ± 32 kBq/g. Interestingly, this value remained similar 3 days after injury (158 ± 10 kBq/g). Noradrenaline uptake was potently inhibited by the application of the noradrenaline reuptake inhibitor nisoxetine at a concentration of 1 μ M during the preincubation and labeling periods, resulting in an average radioactivity of 37 ± 9 kBq/g, a 75% decrease compared to the control group. 3 days after injury this inhibition was significantly less effective (61 ± 16 kBq/g), resulting in a 62% decrease.

[³H]noradrenaline release in the control experiments

In the fractional release experiments the average release during a 200 sec resting period was $0.67 \pm 0.13\%$ of the total radioactivity content (FRR₁). Electrical stimulation caused an additional [³H]NA release of $2.73 \pm 0.68\%$ (FRS₁), which slowly decreased to its resting value. In response to the second electrical stimulation the increase was less pronounced (FRS₂ = $1.94 \pm 0.57\%$) and the FRS₂/FRS₁ ratio was 0.73 ± 0.21 .

The effect of spinal cord injury on [³H]noradrenaline release

Following spinal cord injury both the resting and stimulated [³H]NA release increased significantly. FRR₁, which represents the resting release, was $0.81 \pm 0.09\%$ 1 day after injury, $0.81 \pm 0.12\%$ 3 days after injury and $1.01 \pm 0.46\%$ 14 days after injury. All three are significantly higher than the control value. FRS₁, which represents the stimulated release, was $3.01 \pm 0.95\%$ 1 day after injury, $3.21 \pm 0.74\%$ 3 days after injury and $3.40 \pm 0.95\%$ 14 days after injury. The value measured 3 days after injury was significantly higher than the control value.

These results suggest that 3 days after spinal cord hemisection, there is a 20% increase in the resting release and an additional 18% increase in the stimulated release of noradrenaline.

The effect of uptake inhibition on [³H]noradrenaline release

In half of the fractional release experiments, 1 μ M nisoxetine was added to the perfusion solution starting at the 8th fraction to inhibit [³H]NA reuptake. Thus, after the second electrical stimulation, more transmitter remained in the superfusion solution, resulting in a significant increase in the measured [³H]NA release in all groups.

However, nisoxetine inhibited noradrenaline uptake less effectively after spinal cord injury. While nisoxetine increased FRS₂ by 73% in the control group, this increase was only 61% 1 day after injury and 37% 3 days after injury, the latter being significantly different compared to the control group.

The effect of ion channel blockage on [³H]noradrenaline release

When the voltage dependent Na⁺ and K⁺ channel blocker lidocaine (5 mM) was added to the perfusion solution starting at the 8th fraction, the resting [³H]NA release increased significantly, reaching its maximum at the 13th fraction, then slowly decreased until the end of the experiment. The FRR₂/FRR₁ ratio was 5.22 ± 0.20 ($p < 0.001$). Stimulated release on the other hand was inhibited.

The selective Na⁺ channel blocker tetrodotoxin (1 μM) did not affect resting release, but almost completely inhibited stimulated release. The FRS₂/FRS₁ ratio was 0.19 ± 0.05 , which is one fourth of the control value ($p < 0.001$).

The selective K⁺ channel blocker 4-aminopyridin (300 μM) markedly increased both the resting and stimulated release of [³H]NA (FRR₂/FRR₁ = 5.02 ± 0.17 ; FRS₂/FRS₁ = 3.40 ± 0.24 ; $p < 0.001$).

[³H]noradrenaline uptake and release in the dental pulp

The [³H]NA uptake of human dental pulp following 30 min [³H]NA labeling was 116 ± 69 kBq/g. When noradrenaline release reached its stable resting value, the dental pulp released $1.66 \pm 0.46\%$ of its total [³H]NA content in a 300 sec fraction (FRR₁). Following a 100 sec electrical stimulation, the release (FRS₁) was $5.86 \pm 1.87\%$. After the second stimulation, the release was smaller (FRS₂ = $4.60 \pm 1.42\%$), then returned to its resting value.

CONCLUSIONS

- 1) Our study is the first to measure the changes in noradrenaline release after spinal cord injury, which was simulated by hemisection in rats. After 3 days, noradrenaline release from the decentralised nerve terminals increased significantly, both during resting and stimulated conditions. Electron microscopy revealed an overall degeneration of the tissue.
- 2) The noradrenaline reuptake inhibitor nisoxetine lost its efficiency after spinal cord injury.

We conclude that following spinal cord injury, the increased concentration of noradrenaline exerts neurotoxic effect via its metabolites and its enhancing effect on glutamate release.

- 3) We characterised the changes in noradrenaline release in the spinal cord after the application of lidocaine and selective Na⁺ and K⁺ channel blockers. Na⁺ channel blockage inhibited stimulated release, and K⁺ channel blockage increased both resting and stimulated release. Lidocaine blocked both ion channels, and significantly increased noradrenaline release, which might contribute to its anaesthetic properties, but also to its neurotoxic side effects.

- 4) Our measurements on human dental pulp showed that the noradrenergic terminals in the pulp can be effectively stimulated, and the characteristics of noradrenaline release are similar to that of neural tissue. Thus, the experimental procedure could be used to study the effects of certain pharmacological and pathological conditions on neurotransmitter release in the human dental pulp.

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