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Effect of neurotropin on cerebral edema, calcium and other elements in mice subarachnoidally injected with carrageenan

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Abstract

Neurotropin, an inhibitor of the kallikrein-kinin system, was therapeutically i.p. administered to mice with brain inflammation induced by subarachnoidal injection of carrageenan. Brain water content was determined by the wet/dry weight ratio. The concentrations of cerebral Ca, K, Mn, Fe, Cu, Zn, Se, Rb, and Sr were measured by particle-induced X-ray emission. It was found that neurotropin dose dependently reduced brain water content. The mean concentration of cerebral calcium was significantly lower in the neurotropin-treated group than that in the non-treated group, suggesting less cell damage. Since it has been reported that dexamethasone and some prostaglandin inhibitors have no effect on brain swelling in this model and that, in contrast to these drugs, neurotropin has only a weak inhibiting activity on carrageenan-induced paw swelling, it is hypothesized that the kallikrein-kinin system is differently implicated in cerebral and peripheral inflammation.

Keywords: Brain edema; Carrageenan; Calcium; Kallikrein-kinin; Neurotropin; Particle-induced X-ray emission

1. Introduction

Neurotropin is a non-protein fraction extracted from inflamed skin of rabbits inoculated with vaccinia virus. This drug has been widely used for a long time as an analgesic (Ono, 1981) and an anti-allergic (Okuda, 1979) in Japan.

Neurotropin was recently shown to inhibit the kallikrein-kinin system. Such inhibiting properties were demonstrated both in vitro on the activation of the human plasma kallikrein-kinin system (Nishikawa et al., 1992) and in vivo on the release of bradykinin following noxious stimuli in rats (Ohara et al., 1988) without affecting the concomitant release of histamine, serotonin and prostaglandin E_2. Bradykinin has been implicated in the pathogenesis of vasogenic brain edema, either directly (Kamiya et al., 1993; Raymond et al., 1986; Unterberg and Baethmann, 1984; Unterberg et al., 1986) or by stimulating the phospholipases A_2 and C, leading to arachidonic acid release with free radical and eicosanoid formation, which also contribute to the formation of vasogenic brain edema (Hsu et al., 1990).

In a search for its effects on this pathology, neurotropin has been shown to significantly reduce brain swelling in an experimental model of brain infarction in mice (Sprumont et al., 1993). In a randomized double-blind controlled trial in 220 patients with acute ischemic stroke, neurotropin significantly improved clinical outcome and neurological deficit; it also decreased the size of the infarct and edema zones measured by CT-scan imaging (De Reuck et al., 1994b). In those patients with acute middle cerebral artery infarcts, neurotropin, compared to placebo, lowered the mortality rate (De Reuck et al., 1994a). In the same clinical trial, a post-mortem study showed a significantly lower increase in water content within the infarcted white matter of neurotropin-treated patients compared to that of placebo-treated patients, while the latter demonstrated a higher increase in Ca concentra-

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tion and a more marked decrease in K, Rb and Cu (De Reuck et al., 1993).

The aim of the present study was to test the neurotropin effects on a model of carrageenan-induced brain edema (Gamache et al., 1986). In this model, immunocytochemical methods had previously demonstrated the abnormal presence of bradykinin-like material in cerebral perivascular zones, which was reduced by neurotropin (Kaelin et al., 1991).

The human post-mortem study in recent cerebral infarcts indicated that neurotropin not only reduced brain water content but also altered the concentration of intracytoplasmic elements (especially Ca, K, Rb and Mn). These elements were thus measured in the present investigation as there is a correlation between the brain content of these elements and ischemic damage (Chen et al., 1987; Duflou et al., 1989).

2. Materials and methods

2.1. Animals

The experiments were performed on 52 male NMRI mice from our own stock at the Institute of Anatomy and Special Embryology at the University of Fribourg. They were aged 60–90 days and weighed 30–40 g (mean = 34.804, S.D. = 1.828).

2.2. Intraventricular injection procedure

All subarachnoid injections were administered according to a described method (Gamache et al., 1986). In brief, each animal was anesthetized by an intraperitoneal injection of 55 mg/kg Nembutal, and a constant volume of 5 μl of either normal saline or 10 mg/ml λ-carrageenan (Sigma Chemical Co., St. Louis MO, USA), freshly made solution in distilled water, was injected through the skull. The selected dose of carrageenan has been reported to give the maximum response in brain water increase (Gamache et al., 1986).

2.3. Treatment

All treatments were administered by intraperitoneal route at a constant volume of 1 ml. The following substances were injected: normal saline and neurotropin (Nippon Zoki Pharmaceutical Co., Osaka, Japan) at doses of 8.3, 12.5 or 25 mg/kg diluted with normal saline solution. The therapeutic injection took place about 2 h after carrageenan administration.

2.4. Experimental timing

Starting from time zero (T_0) the average timing of each experiment was as follows, with the elapsed times from T_0 given in min: $T_0 - T_{10}$, subarachnoidal injection of carrageenan or saline; $T_{130} - T_{135}$, administration of neurotropin or saline; $T_{255} - T_{270}$, vascular rinsing and brain weighing.

The timing of treatment administration was determined according to the time course of the brain vascular permeability response, which starts at 2 h and exhibits its peak 4 h after carrageenan subarachnoidal injection (Gamache et al., 1986).

2.5. Brain water determination

The animals were given a lethal intraperitoneal injection of 200 mg/kg Nembutal about 2 h after treatment. Phosphate-buffered saline was perfused over the left ventricle for 2 min. The brain was then removed and weighed for wet weight (Ww) in a tared crucible on a Mettler A30 precision balance. The opened crucible was placed with its content at 110°C in an oven for 16–18 h, then closed and weighed again for dry weight (Dw). Brain water content (BW) was calculated as a percentage as follows: $BW = (Ww - Dw/Ww) \times 100$.

2.6. Determination of elements by particle-induced X-ray emission

Five carrageenan-injected mice and four carrageenan-injected neurotropin-treated (25 mg/kg) mice were randomly selected from the above-mentioned experimental groups and analyzed blind.

Particle-induced X-ray emission was used to measure calcium and eight other elements (K, Mn, Fe, Cu, Zn, Se, Rb, and Sr) in the selected dried brains. The brains were prepared for the elemental analysis by using a procedure described by Duflou et al. (1987). The procedure involved acid digestion with concentrated HNO_3 in a closed Teflon vessel, and four specimens were prepared and analyzed for each brain.

The method used, experimental setup and quantification procedures are described in detail elsewhere (Duflou et al., 1987; Maenhaut et al., 1980, 1987; Maenhaut and Raemdonck, 1984; Maenhaut and Vandenhaute, 1986). In brief, the specimens were bombarded with a 2.4-MeV proton beam of 0.5 cm^2. This gave rise to the emission of characteristic X-rays, which were measured with an energy-dispersive Si(Li) detector system. A 660-μm thick Mylar absorber was placed in front of the detector in order to absorb the low energy X-rays originating from the matrix elements in the specimen. The beam current at the specimen was typically between 100 and 200 nA, the count rate was kept below 1500 cps, and the preset charge was 90 μC. The intensities of the characteristic X-rays in the particle-induced X-ray spectra were obtained by means of the nonlinear least-squares fitting program AXIL

(Maenhaut and Vandenhaute, 1986). After applying corrections for proton beam energy degradation and X-ray attenuation in the specimen, elemental concentrations in $\mu g/g$ dry weight were finally obtained.

Favorable features of the technique are its multielement character, the speed of the analysis (analysis time of 5–10 min), the very limited sample requirement (a few mg are sufficient) and the detection limits down to 0.1 $\mu g/g$ for soft biological tissues. The precision and accuracy of our particle-induced X-ray analysis procedures were thoroughly examined through comparisons with neutron activation analysis (Duflou et al., 1987) and by the analysis of certified reference materials (Maenhaut et al., 1987). It was concluded that the accuracy is better than 5%.

2.7. Statistical analysis

The differences between brain water contents of the experimental groups were investigated by one-way analysis of variance followed by Newman-Keuls comparison on matched pairs. Differences were considered to be significant if they reached the 0.05 level. A correlation and linear regression analysis was also performed.

The concentrations of calcium and eight other elements in the brains of the neurotropin-treated animals were compared to those of the nontreated animals; the nonparametric Mann-Whitney U-test was used to establish whether the difference between both groups was statistically significant. This was considered to be so when the two-tailed probability (P) of the data being from the same population was less than 0.05.

3. Results

The effects of neurotropin treatment on carrageenan-induced brain edema compared to control treatment are indicated in Fig. 1. Therapeutic administration of neurotropin dose dependently reduced the increase in brain water due to carrageenan injection and, at the dose of 25 mg/kg, brought it almost to normal.

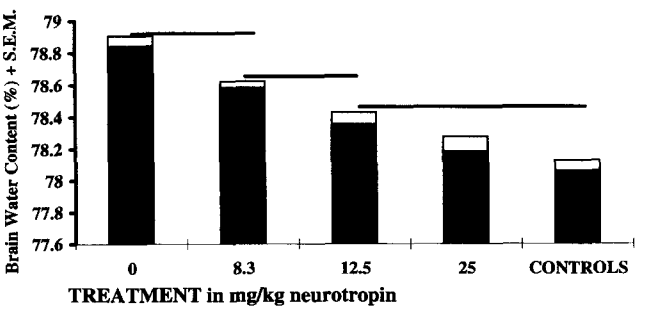

Fig. 1. Histogram of the brain water content (% of wet weight), mean values and standard errors (grey and clear vertical bars), in the five experimental groups. Significantly ($P < 0.05$) different groups are indicated by a horizontal thick line. Adjacent values not covered by such a line are not significantly different.

Table 1
Concentrations (in $\mu g/g$ dry weight) of cerebral elements in five normal animals and in four carrageenan-injected mice treated with 25 mg/kg neurotropin

Element	Control Mean ± S.E.M.	Neurotropin-treated Mean ± S.E.M.
K	17920 ± 320	18220 ± 340.00
Ca *	1030 ± 500	246 ± 9
Mn	1.89 ± 0.06	1.9 ± 0.05
Fe	53.5 ± 1.8	54.3 ± 3.9
Cu	18.4 ± 0.4	18.9 ± 0.4
Zn	87.8 ± 0.9	87.0 ± 1.6
Se	0.67 ± 0.05	0.72 ± 0.05
Rb	13.2 ± 0.2	136 ± 0.3
Sr	0.4 ± 0.2	< 0.4 -

* Elemental concentration statistically different ($P < 0.014$) between both groups.

A correlation analysis was performed on all experimental animals treated with either normal saline or up to 12.5 mg/kg of neurotropin (NEU). A highly significant ($t = 5.45$ for 30 freedom degrees; two-tailed $P < 0.0001$) correlation coefficient (Pearson's r) of 0.7053 was observed. The corresponding linear regression equation was as follows: brain water content (%) = 78.859 − 0.0375 NEU dosage (in mg/kg).

The elemental concentrations in the brain of carrageenan-injected animals are reported in Table 1. It was observed that in the carrageenan-injected neurotropin-treated group the mean concentration of brain calcium was significantly lower than in the carrageenan-injected saline-treated group. For the other elements, no significant difference was found between the two groups.

4. Discussion

Carrageenan-induced inflammation is a standard animal model and a reduction in inflammation is considered to be highly predictive of anti-inflammatory drug activity (Otterness and Bliven, 1985). An essential involvement of both prostaglandin and kallikrein-kinin systems has been demonstrated in the carrageenan-edema induced in rat hind paw and peritoneal cavity (Damas et al., 1990; Ferreira et al., 1974, Oh-ishi et al., 1989).

In the mouse brain, the inflammation induced by carrageenan subarachnoidal injection also increased arachidonic acid metabolism (Gamache et al., 1986), while abnormal fringes of extracellular bradykinin-like immunoreactive material were immunocytochemically detected around many cerebral blood vessels (Kaelin et

al., 1991). In this brain edema model, prostaglandin inhibitors such as indomethacin and ibuprofen had no effect on either cerebral swelling or vascular permeability whereas dexamethasone only inhibited the permeability increase (Gamache and Ellis, 1986). But the same drugs strongly reduced the inflammation locally produced by carrageenan injection outside the central nervous system (Henriques et al., 1987; Otterness and Bliven, 1985). In contrast, neurotropin did not suppress the occurrence of carrageenan-induced paw swelling in rats, but only accelerated its resolution (Takeuchi et al., 1987). In mouse brains, however, our present experiments demonstrated that neurotropin dose dependently reduced carrageenan-induced cerebral edema. This observation can be compared with the previously reported inhibition by neurotropin of the release of bradykinin-like immunoreactive material in the same brain edema model (Kaelin et al., 1991).

In the same experimental model, Gamache and Ellis (1986) considered blood-brain barrier impermeability as a possible cause for the lack of effect of prostaglandin inhibitors on brain edema but the difference between dexamethasone activities upon cerebral and peripheral inflammation remains so far unexplained.

From our results, we are tempted to raise a new hypothesis. Reviewing the mechanism and role of kinin formation in inflammatory disorders, Proud and Kaplan (1988) stated that 'kinins are generated by both the plasma kinin-forming system and the tissue kallikrein system. The importance of the tissue kallikrein system depends upon secretion of the active form of the requisite enzyme in the presence of a source of kininogen. The plasma system will be activated secondary to inflammation initiated by some other process. There may be endothelial or epithelial damage exposing connective tissue. Plasma leakage caused by release of some other permeability factors (including kinin made by tissue kallikrein) would thus lead to activation of the plasma kallikrein-kinin cascade in many forms of inflammation'. Nevertheless, the extent to which kinins are involved in the inflammatory process remains to be determined (Katzung, 1987). Since neurotropin has been observed to inhibit plasma kallikrein-kinin cascade more strongly than tissue kallikrein-kinin cascade (Nishikawa et al., 1992), the difference between the neurotropin activity upon carrageenan-induced cerebral and peripheral swelling might be explained by a different involvement of the plasma kallikrein-kinin system at both locations. It must be pointed out that the blood-brain barrier and skull volume are physical limitations which do not exist in peripheral soft tissues. These can therefore more freely respond to the various inflammation mediators involving both plasma and tissue kallikrein-kinin systems as well as prostaglandins. Within the brain, however, because of the physical limitations, the vascular content and thus the plasma kallikrein-kinin system would play the main role in maintaining cerebral edema: neurotropin, by specifically inhibiting the plasma kallikrein-kinin cascade, effectively reduces brain edema.

This hypothesis would also tentatively explain why the activity of dexamethasone on carrageenan-induced edema is lower in brain than in peripheral soft tissues, since kallikrein-kinin cascade inhibition does not contribute to the main anti-inflammatory actions of dexamethasone (Goodman and Gillman, 1992). It is interesting to point out that similar differences between dexamethasone and neurotropin activities on brain edema were reported in ischemic stroke patients (Norris and Hachinski, 1986; De Reuck et al., 1988, 1994b).

In our elemental experiments, the lower calcium concentration in the brain of the neurotropin-treated animals is consistent with an inhibition of the kallikrein-kinin cascade. Bradykinin stimulates receptors acting through the inositol phosphate pathway, activation of which leads to an elevation of intracellular calcium (Bareis et al., 1983). But the lower calcium concentration could also be a consequence of the neurotropin anti-edema effect since a correlation between brain calcium concentration and rate of ultimate brain cell damage has been demonstrated in ischemic conditions (Chen et al., 1987).

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