Effects of the noradrenaline neurotoxin
N-2-chloroethyl-N-ethyl-2-bromo-benzylamine hydrochloride (DSP4) on the blood-brain barrier
An experimental study in the mouse using protein tracer and density determination techniques*

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Summary. Cerebral microvessels receive a noradrenergic innervation originating from the locus coeruleus. Previously, many studies have tried to elucidate the role of the central noradrenergic innervation on the blood-brain barrier (BBB). Many of them are based on chemical destruction of the innervation by local injection of 6-hydroxydopamine (6-OHDA) or physical injury to the locus coeruleus. Such methods are not selective and the results reported are contradictory. We have treated mice with a single i.p. injection of the compound, N-2-chloroethyl-N-ethyl-2-bromo-benzylamine hydrochloride (DSP4). This substance induces a selective noradrenaline depletion and, unlike 6-OHDA, it can pass into the brain after an i.p. injection. The animals were allowed to survive for 6 h to 60 days and the BBB was investigated with i.v.-injected horseradish peroxidase (HRP). Brain density values were also determined to find out if edema developed. The light microscopic distribution of HRP in the brain of DSP4-treated animals did not differ from that in control mice, i.e., there were no signs of increased BBB permeability to this protein tracer caused by DSP4. Density determinations revealed statistically significant reduced values in cerebrum (P < 0.005) and rhombencephalon (cerebellum) (P < 0.0005) of animals given 100 mg/kg body wt. of DSP4 indicating development of edema. A minor drop in density of the rhombencephalon (cerebellum) (P < 0.05 at 48 h) and of the cerebrum (statistically not significant) appeared when 50 mg/kg body wt. of DSP4 was injected. Our findings indicate that the BBB to proteins maintains its function but that edema, likely composed of an ultrafiltrate from the blood, will develop after an injection of DSP4. In view of its selective degenerative action on the noradrenergic central neurons, this kind of brain edema is probably a direct consequence of abnormal noradrenergic innervation of the cerebral blood vessels. Our observations are thus in line with the assumption that the noradrenergic innervation influences endothelial permeability in the central nervous system. Alternative pathogenetic mechanisms are discussed.

Key words: DSP4 — Noradrenaline — Blood-brain barrier — Brain edema — Brain density

The central nervous system (CNS) is innervated by two ascending noradrenergic pathways, i.e., the locus coeruleus system and the lateral tegmentum pathways [26, 29, 45]. Cerebral microvessels receive innervation from neurons located in the locus coeruleus and also from the peripheral autonomic nervous system [13, 24, 35]. The central neurons terminate on capillary endothelial cells and on pericytes [35] and adrenergic receptors are present on cerebral microvessels [11, 15, 44].

Earlier observations indicate that several functions of the cerebral vascular endothelium may be modified by the noradrenergic innervation [9, 25, 34]. It may influence blood flow, vascular resistance [33, 34] and the blood-brain barrier (BBB) but the results of the studies so far presented are contradictory. Thus, stimulation of cervical sympathetic neurons might protect the BBB in acute hypertension [14] but cervical sympathectomy does not lead in itself to BBB disruption [8]. However, electrical and chemical stimulation of locus coeruleus neurons increases BBB permeability to water [33, 34]. Chemical injury to the central...
catecholaminergic innervation of cerebral vessels by intracerebral or intraventricular administration of 6-hydroxydopamine (6-OHDA) increases the vulnerability of the BBB to acute hypertension [1]. Unilateral injection of this compound into the locus coeruleus causes a significant ipsilateral increase in albumin leakage into the brain in norepinephrine-induced hypertension and in seizures induced by bicuculline [10].

The neurotoxin, 6-OHDA, must be injected directly into the brain in order to exert its effects on the neurons of the locus coeruleus since it can not pass the BBB. In such experiments it is hard to control its delivery into the brain and the injection may lead to several nonselective degenerative phenomena in the parenchyma [3, 4, 20]. The introduction of the haloalkylbromobenzylamine N-2-chloethyln-ethylbromobenzylamine hydrochloride; (DSP4) [37, 39] into this field of research appears to open a new way of studying the influence of the noradrenergic innervation of the cerebral vessels since this compound can pass the BBB after an i.p. injection [7] and its effect appear to be highly selective to noradrenaline containing terminals [2, 20-22]. The degenerative action after a single i.p. injection of the neurotoxin leads to a longlasting noradrenaline depletion of nerve terminals [22].

This investigation was carried out to expand our knowledge of the relationships between the innervation of the cerebral blood vessels and the function of the BBB. The specific purpose of our study was to find out if a single i.p. injection of DSP4 will influence the BBB to a protein tracer and lead to the formation of edema. Such an experimental approach appears to have several advantages over local 6-OHDA injection or physical destruction of the locus coeruleus. Experimental models based on unilateral injections into the locus coeruleus of 6-OHDA will cause unspecific lesions and noradrenaline containing neurons located outside the ipsilateral locus coeruleus will be spared. In fact, some CNS regions such as the posterior half of the cerebellar cortex, the medial geniculate body and the inferior colliculus are bilaterally innervated from the locus coeruleus. The anterior half of the cerebellar cortex receives its main innervation from the ipsilateral locus coeruleus and other noradrenergic nerve cell populations also contribute to the ipsilateral innervation [24]. It is thus reasonable to assume that the delivery of the neurotoxin DSP4 by i.p. injection will induce a more marked central noradrenaline depletion than local unilateral 6-OHDA injection.

Materials and methods

The experiments were carried out on 89 adult male albino mice of the NMRI strain weighing 29-32 g (ALAB, Sollentuna, Sweden). The animals were housed 5 per cage and had free access to food pellets and tap water.

DSP 4 injections

The alkylation agent DSP4 is structurally related to the alpha-1 adrenoreceptor blocker phenoxbenzamine [16]. However, DSP4 is about 20 times more potent [38].

Forty-six conscious mice received a single i.p. injection of DSP4 at 50 mg/kg body wt. dissolved in 0.5 ml isotonic saline. The mice were thereafter allowed to recover for 6, 24, 48 h or 7, 14, 28, 60 days before sacrifice.

We chose a dose of 50 mg/kg body wt. since previous investigations had shown that this dose induces a marked noradrenaline depletion in several CNS regions [16, 22]. Apart from these 46 animals, 7 other mice were injected with 100 mg/kg body wt. of the neurotoxin and allowed to recover for 48 h. This increased dose further potentiates the inhibitory action of DSP4 on uptake of noradrenaline in mouse CNS [38]. Special care was taken to inject only freshly prepared DSP4 solutions since it is known that DSP4 will spontaneously be transformed to a still potent derivate, which is, however, unable to pass the BBB [16, 39].

It is known that conscious rats are more resistant than anesthetized ones to BBB dysfunction induced by acute hypertension [18]. This may be explained by a higher state of activity in neurons of the locus coeruleus in the conscious rats as compared to the anesthetized animals (cf. [19]). We therefore anesthetized seven DSP4-treated animals and six control mice with barbiturate preceding the horseradish peroxidase (HRP) injection to prolong the unconscious state. The barbiturate anesthetized animals awoke after approximately 2 h, whereas the mice given ether anesthesia were conscious within a few minutes. The two groups were compared with regard to differences in protein extravasation in the brain.

HRP injections

Three or six hours before sacrifice 21 DSP4-treated mice, anesthetized with ether or sodium pentobarbital (Nembutal), 60 mg/kg body wt., were given a single i.v. injection of 10 mg/animal of HRP (Sigma, type II) dissolved in 0.2 ml isotonic saline. Nineteen mice injected with 0.5 ml isotonic saline instead of DSP4 were also injected with HRP and served as controls.

Fixation of brain tissue was carried out in the following way. Under ether or Nembutal anaesthesia the thorax was rapidly opened and a cannula was inserted in the left ventricle. An outlet of blood and perfusate was created by opening the right atrium. Perfusion fixation was started with a brief saline washout, followed by a fixative for 30 min. The perfusion pressure was 110 mm Hg and fixation was carried out at room temperature.

The fixative was composed of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4. The animals were perfused for 30 min with 10% sucrose in the same buffer at 4°C. The brains were removed and stored in the sucrose buffer solution at 4°C until ready for HRP histochemistry, but not for more than 24 h [36]. Coronal tissue blocks were removed as demonstrated in Fig 1.

Forty-micrometer-thick frozen sections were cut from each tissue block on a cryomat and histochemistry for HRP activity was carried out using the sensitive tetramethylbenzidine (TMB) technique described by Mesulam [28]. All slides were examined by light microscopy.

Density determinations

Reduction of brain density values reflects the formation and also changes in the magnitude of brain edema. Therefore, deter-