Evaluation of Possible Spinal Neurotoxicity of Clonidine

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ABSTRACT

An experimental investigation was carried out concerning the potential neurotoxic effects of clonidine. For this purpose intrathecal or extradural catheters were implanted in dogs. Clonidine at a dose level of 25 ug/kg or 12.5 ug/kg, or placebo, was then administered via the catheter once daily for 14 consecutive days. The spinal cord and the nerve roots were then taken for neuropathological analysis.

Observation of the neurological behaviour of the animals and the results of the morphological investigation support the conclusion that clonidine under these circumstances does not have any significant neurotoxic effects.

INTRODUCTION

The existence of several endogenous systems which modulate nociceptive information at the spinal cord level has previously been demonstrated in experimental animal. The endorphine system (1,2), the analgesic capacity of which is used in clinical praxis by administration of opiates systemically or close to the central nervous system (CNS) is the best known example of this group. Another pain-modulating system effective at the level of the spinal cord uses norepinephrine as the transmittor substance (3). Norepinephrine applied to the spinal cord in animals gives rise to a segmental dose-dependent analgesia reversible with specific alpha-adrenoreceptor blockers (4).

Clonidine, an imidazole compound, also exhibits analgesic properties (5), presumably by acting as an agonist on the presynaptic alpha-2-adrenoreceptors (6) in the spinal cord (7), as well as in supraspinal parts of the CNS (8,9). An analgesic effect has been demonstrated upon intravenous administration of clonidine to patients with postoperative pain (10). Thus, in addition, clonidine administered intrathecally gives segmental dose-dependent analgesia com-

parable to intrathecal morphine (4). Intrathecal co-administration of sub-analgesic doses of morphine and ST-91, a clonidine analogue, also gives potent analgesia. A true potentiation seems to occur as well as a markedly reduced tendency to develop tolerance (11) to the individual substances. The possibility of modulating noxious impulses at the dorsal horn is of considerable interest in clinical medicine. Clonidine might in the future be used in man for this purpose. Both the intrathecal and epidural route of administration might then be used. However, before it is tested clinically, the toxicity of the drug must be known. The systemic toxicity of clonidine by the oral and intravenous route is well documented (12). Neurotoxicological studies where clonidine has been administered chronically by the intrathecal or the epidural routes have not been reported.

The present experiments were carried out to examine if clonidine administered by the spinal route has any severe adverse effects on the spinal cord and nerve roots which could be detected by a neuropathological analysis at the light microscopical level.

MATERIAL AND METHODS

Intrathecal catheters were implanted in female beagle dogs. The animals were then observed for seven days. Only those who were completely neurologically unaffected by the catheter implantation procedure took part in the study (n=5). Clonidine at either of two dose levels, 25 ug/kg or 12.5 ug/kg, or placebo was then administered in the catheter, once daily, for 14 consecutive days. Two dogs received the higher dose, two the lower and one received placebo. One dog served as control for the perfusion technique used for tissue fixation, and no catheter was implanted in this animal (n=1) (Table 1).

Table 1. Given doses of clonidine and catheter position

	Dose	Position of catheter
Dog no 1:	Clonidine 25 ug/kg/day	Intrathecal
Dog no 2:	Placebo	Extradural
Dog no 3:	Clonidine 12.5 ug/kg/day	Intrathecal
Dog no 4:	Clonidine 25 ug/kg/day	Extradural
Dog no 5:	Clonidine 12.5 ug/kg/day	Extradural
Dog no 6:	No catheter	

After each injection the dogs were observed by a veterinarian for signs of abnormal behaviour, including signs of neurological deficit. The body weight was registered at day 1 and 14. After 14 days the dogs were sacrificed.

Surgical protocol

For implantation of the intrathecal catheter the animals were anaesthetized with Pentothal i.v., the trachea was intubated and anaesthesia was maintained with a mixture of oxygen and N_2O, 50/50, to which 0.5 % halothane was added. The ventilation was assisted. The dogs were placed with head flexed forward. Following aseptic procedures the atlanto-occipital membrane was exposed and the dura cut rostro-caudally in the midline. A catheter (Verdural[R] epidural catheter 0.8 mm ext. diameter) was inserted in the subdural space and directed caudally until its tip was considered to be located in the lumbar region. The proximal end of the catheter was sutured to the skin on top of the head of the animal. The free end of the catheter was connected to an antibacterial filter (Transcodan microfilter[R], 0.2 um) through which the injections later were given. Tape was used to protect the free end of the catheter and filter. The procedure is a modification of the method described for rats by Yaksh et al. (13).

Injection protocol

All test drugs were given in volumes of 1 ml. The catheter system and anti-bacterial filter were primed with the test drug.

Drugs

Drugs used were clonidine HCl (Boehringer Ingelheim), pH 4.0, 289 mosm/kg H_2O; and 0.9 % NaCl, pH 6.0, 280 mosm/kg H_2O.

Tissue preparation

The animals were anaesthetized in the same way as for catheter implantation. After thoracotomy a large-bore catheter was introduced into the left ventricle. After pretreatment with heparin 500 IU i.v. and controlled ventilation with 100 % oxygen, 2 litres of Ringer's solution were infused under pressure through the left ventricle, to exanguinate the animal. The blood escaped through an incision in the right ventricle. Immediately after the Ringer infusion, without interruption of the stream, 2 litres of a buffered 2 % solution of paraformaldehyde (pH 7.4) followed without letting air into the system. Fifty ml of buffered paraform - 2 % glutaraldehyde were then injected through a needle inserted percutaneously through the atlanto-occipital membrane to immerse the already perfused spinal cord. An outlet for the solution infused, to avoid abnormal pressures around the cord, was made by inserting a large bore needle percutaneously beside the needle mentioned above.

Five dogs were necropsied 1 h after sacrifice (no. 1, 2, 3, 4, 6). Dog no. 5 was necropsied within 12 h after sacrifice. A routine autopsy was performed on all dogs. The brain, spinal cord (including nerve roots) and sciatic

nerves were removed segment by segment and immersed in 2 % paraformglutar-
aldehyde solution and stored at +4^{0}C. The tissues were sampled from all cervi-
cal, thoracic and lumbar spinal cord segments in all dogs. Tissues from brain,
spinal cord (including nerve roots) and sciatic nerves were paraffin embedded,
sectioned and stained with hematocylin-eosin and luxol fast blue. Light micro-
scopy was used for examination of the tissue samples using coded sections.

RESULTS

The body weight did not change significantly in any dog during the 14 day
trial period.

Signs of sedation were noticed in the two dogs receiving the high clonidine
doses for about 1 h after injection. No other changes in general appearance or
neurologic behaviour could be detected by observation of the animals during
the entire period.

No gross lesions were noted in the spinal cord and the brain at autopsy.

Tissue reaction to the catheter and location of catheters

Multiple cross sections from the cord revealed that the catheter in dog
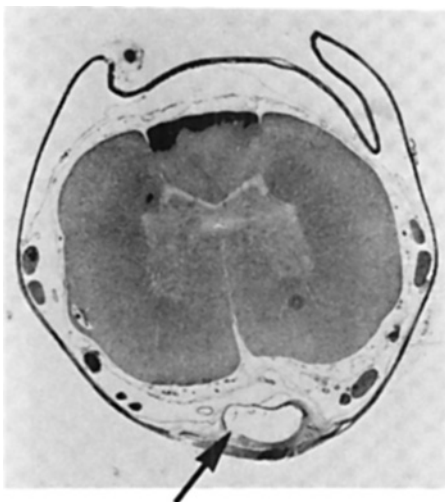no. 1 and 3 was located in the subarachnoidal space (Fig. 1).

Fig. 1

Cross section of the thoracic
spinal cord (dog no. 1). The
catheter is located ventrally in
the subarachnoidal space.

In the remaining dogs, no. 2, 4, 5, including the control the position of
the tip of the catheter was immediately outside the dura. The tip of the
catheter was located between T_{13} and L_4. It was surrounded by a narrow zone
composed of granulation tissue and connective tissue. The amount of granula-
tion tissue varied from segment to segment between individuals. In dog no. 1
the catheter caused in some areas a marked impression of the spinal cord

parenchyma; in dog no. 3 it caused a very mild impression.

Spinal cord and nerve roots

As shown in Fig. 1 the spinal cord looked normal in all the dogs apart from
a mild unilateral swelling of the peripheral part of the cord along the
course of the catheter (Fig. 2). The long tracts did not show any signs of in-
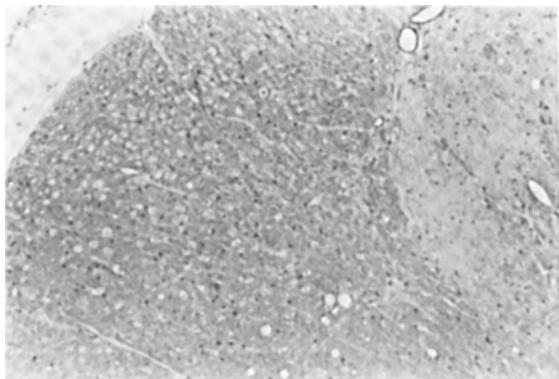jury and the ventral motor neurons were normal (Fig. 3a, 3b).

Fig. 2
Lumbar spinal cord from dog
no. 3. Dorsal area of the
lateral funiculus including
dorsal spinocerebellar and
lateral corticospinal tracts.
Notice mild swelling of par-
ticularly the peripheral part
of the cord.

The intradural parts of the roots were entirely normal (Fig. 4) except for
a small area in a lumbar nerve root of dog no. 1. In this part there was a
group of closely packed fibers which apparently had lost their myelin sheaths.

A few neurons in the cranial spinal ganglia of all the dogs showed signs of
injury with proliferation of satellite-capsule cells. It should be emphasized,
however, that the changes were slight and were restricted to areas that had
been in direct contact with the catheter. The areas close to the distal end of
the catheter did not differ histologically from more proximal parts. The scia-
tic nerves revealed no significant microscopic lesions in any of the dogs.

Brain

Multiple sections were randomly selected from the cerebrum and cerebellum.
In all the dogs, including the controls, microscopic examination of the cere-
bral cortex revealed several shrunken eosinophilic neurons with pyknotic
nuclei. They were scattered diffusly within the cortex. They were not accom-
panied by any glial cell reaction and probably occurred as a consequency of
the perfusion with saline prior to fixation. The changes were present also in
the dogs that received neither clonidine, nor catheter. Why the brain cortex
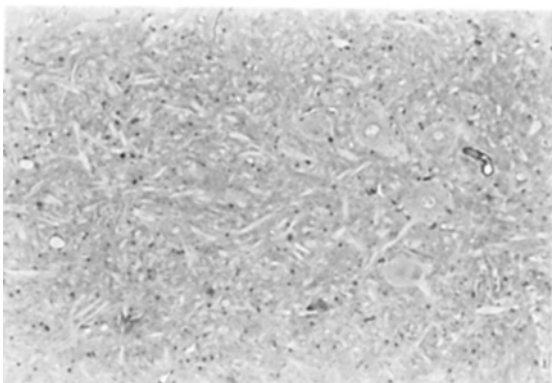came out less well preserved, using this method, remains to be settled.

Fig. 3

Lumbar spinal cord from
dog no. 3. No changes vi-
sible in the ventral horn
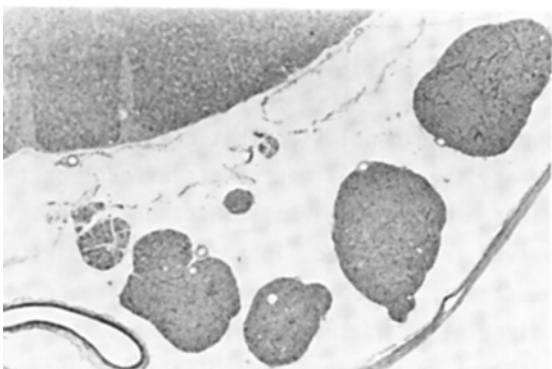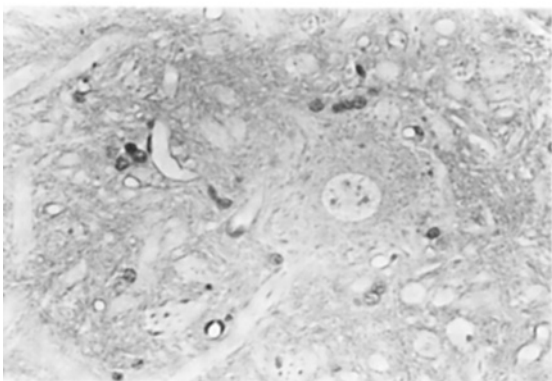(a). Ventral motor neurons
look perfectly normal (b).

Fig. 4

Lumbar area from dog no. 3.
Intradural parts of the
nerve roots have a normal
appearance.

DISCUSSION

The methods of tissue fixation are crucial in detection of the neurotoxic action of a drug. In this study we used buffered glutaraldehyde solution to immerse the spinal cord in situ, after perfusing the animals with paraform-aldehyde. This technique resulted in histological preparations of very good quality from the spinal cord and roots even though the brain appeared less well preserved. Therefore the methods applied should be adequate for the purpose of our experiments. The doses of clonidine were 5 and 10 times higher than the dose normally used in man on other indications. Thus, in humans, 2 ug/kg body weight of clonidine i.v. is known to affect the CNS, as indicated by a blood pressure reduction (12).

No signs of harmful effects of clonidine could be observed in the neurologic behaviour of the animals during the 14 day experiment period. The histological changes noticed in the spinal cords and nerve roots were all along the course of the catheter, indicating a mechanical cause. No signs of diffuse neuronal damage or damage of certain cell types indicating a general toxic effect of the tested substance could be observed. The histological picture of neuronal structures close to the distal end of the catheter, where the concentrations of clonidine were the highest, did not differ from more remote areas.

No difference in the histological picture of the spinal cords and nerve roots was present in either group of animals, whether the catheter was located intrathecally or epidurally. Clonidine is considered to pass easily over the blood-brain barrier (14), and the possible neurotoxicity is of equal interest in both routes of administration.

Intrathecal and epidural administration of clonidine, 12.5 ug/kg and 25 ug/kg, for 14 consecutive days did not give rise to any detectable neurotoxic changes attributable to clonidine. The discrete signs of injury to the spinal cord were also found in the control animal receiving placebo. The lesions are well explained solely by mechanical effects of the implanted catheter.

The findings presented here support the conclusion that clonidine, in the investigated doses, does not have any significant neurotoxic effects on the spinal cord and the intradural parts of the nerve roots. Further neurotoxic studies in other species are, however, recommended. In addition, studies concerning the effect of intra- and extradurally administered clonidine on other parameters, e.g. regional blood circulation of the spinal cord are recommended before trials on humans are carried out on a larger scale. Finally, more detailed neurohistological examinations should be carried out taking advantage of plastic embedding combined with electron microscopy.

REFERENCES

1. Terenius, L. & Wahlström, A.: Search for an endogenous ligand for the opiate receptor. Acta Physiol Scand 94:74-81, 1975.
2. Hughes, J.: Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. Brain Res 88:295-308, 1975.
3. Headly, M.P., Duggan, A.W. & Griersmith, B.T.: Selective reduction by noradrenaline and 5-hydroxytryptamine of nociceptive responses of cat dorsal horn neurons. Brain Res 145:185-189, 1978.
4. Reddy, S.V.R., Maderdrut, J.L. & Yaksh, T.L.: Spinal cord pharmacology of adrenergic agonist-mediated antinociception. J Pharmacol Exp Ther 213: 525-533, 1980.
5. Paalzow, L.: Analgesia produced by clonidine in mice and rats. J Pharm Pharmacol 26:361-363, 1974.
6. Anden, N.E., Graboeska, M. & Strömbom, U.: Different alpha-adrenoreceptors in the central nervous system mediating biochemical and functional effects of clonidine and receptor blocking agents. Naunyn Schmiedebergs Arch Pharmacol 292:43-52, 1976.
7. Spaulding, T.C., Venafro, J.J., Ma, M.G. & Fielding, S.: The dissociation of the antinociceptive effect of clonidine from supraspinal structures. Neuropharmacol 18:103-105, 1979a.
8. Paalzow, G. & Paalzow, L.: Clonidine antinociceptive activity: effects of drugs influencing central monoaminergic and cholinergic mechanisms in the rat. Naunyn Schmiedebergs Arch Pharmacol 292:119-126, 1976.
9. Skingle, M., Hays, A.G. & Tyers, M.B.: Antinociceptive activity of clonidine in the mouse, rat and dog. Life Sci 31:1123-1132, 1982.
10. Gordh, T. & Tamsen, A.: A study on the analgesic effect of clonidine in man. Acta Anaesthesiol Scand suppl 87, 27:72, 1983.
11. Yaksh, T.L. & Reddy, S.V.R.: Studies in the primate on the analgesic effects associated with intrathecal actions of opiates, alphaadrenergic agonists and baclofen. Anesthesiology 54:451-467, 1981.
12. Hoefke, W.: Clonidine. In: A. Scriabine, Ed., Pharmacology of antihypertensive drugs. Raven Press, New York, 55-78, 1980.
13. Yaksh, T.L. & Rudy, T.A.: Chronic catheterization of the spinal subarachnoid space. Physiol Behav 17:1031-1036, 1976.
14. Schmitt, H., Le Douarec, J.C. & Petillot, N.: Antinociceptive effects of some alpha-sympathomimetic agents. Neuropharmacol 13:289-294, 1974.

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