Development of Tolerance to the Antinociceptive Effect of Morphine After Intraventricular Injection

Recently, it has been shown by means of injection of morphine into the ventricular system (VS) and restricted parts of it that the drug acts on structures in the surrounding of the 4th ventricle when inhibiting a nociceptive reaction in rabbits, the licking reaction elicited by electrical stimulation of the tooth pulp[1,2]. Furthermore, in the same species, it was found that the inhibition of the hindleg flexor reflex is brought about by an action of morphine on similar periventricular structures of the caudal brain stem. A direct site of action in the lumbar spinal cord, where the reflex is mediated, seems to be of little importance for the inhibition of this reaction even when the drug is applied systemically[3,4]. The question arose whether, and to what extent, tolerance to the inhibition of these two reactions develops upon repeated intraventricular morphine injection. Up to now, there are only meagre data showing development of tolerance to various actions of morphine when the drug is injected intraventricularly or directly into brain tissue[5-7]. The present investigation shows that upon repeated intraventricular morphine injections considerable tolerance develops to the inhibition of these two nociceptive reactions. This is interesting in view of the fact that the lumbar spinal cord, where the hindleg flexor reflex is relayed, is reached by morphine only in small amounts.

The development of tolerance to morphine following repeated intraventricular administration was compared with the tolerance upon systemic injection. The intraventricular injections were performed in animals with cannulae chronically implanted in the lateral ventricle. The licking reaction was elicited by electrical stimulation of the tooth pulp of the upper incisors. The hindleg flexor reflex was evoked by application of radiant heat to the sole of the rabbit's foot. (For details of these methods see[1,3]). Morphine sulfate (the doses refer to the free base) was injected twice a day (08.00 h and 20.00 h) under aseptic conditions either intraventricularly or i.m. for periods up to 4 weeks. The animals of the control series were injected with saline. Before and during the injection period at one week intervals the antinociceptive effect of i.m. applied morphine was tested (test reaction) in both series at about 10.00 h. On these days, the regular morphine injection in the morning was not given.

Figure 1 shows inhibition of licking reaction in the morphine series in comparison to the saline series. After 1 week of morphine injection (10 mg/kg twice a day) the previously effective test dose of 10 mg/kg i.m. had become almost ineffective. Increasing the test dose to 20 mg/kg and to 40 mg/kg in the later tests, also resulted in only slight effects. Upon intraventricular injection of 2×40 µg/day the tolerance was comparable to the effect of 10 mg/kg/day applied i.m. A significant tolerance also developed for the inhibition of the hindleg flexor reflex (Figure 2). This tolerance, after 14 days, was more pronounced when 40 µg of morphine was applied intraventricularly than when 10 mg/kg was given systemically ($P < 0.05$). It is quite clear that in both tests morphine (when body weight is taken into account) is about 500 times more effective intraventricularly than i.m. A very similar proportion of systemic/intraventricular equieffective doses was found in acute experiments for the antinoceptive activity of morphine[8,9]. This result was not unexpected considering the development of tolerance for the licking reaction. The long persistance of high morphine concentrations in the VS and consequently in the surrounding brain tissue explains this result, and supports the theory that tolerance develops when the receptors are interacting with the drug[10]. In view of the hypothesis of COCHIN and KORNETZKY[10] concerning the involvement of immune processes in tolerance develop-

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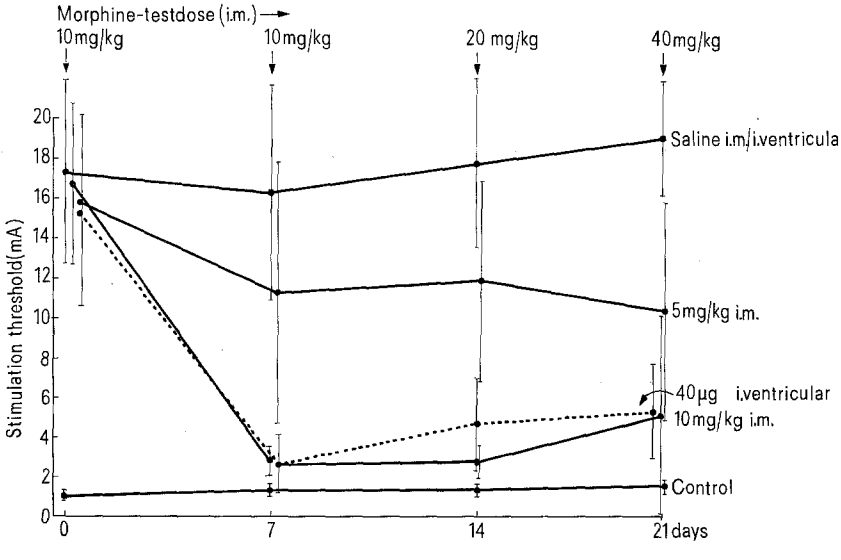

Fig. 1. Development of tolerance to the morphine-induced inhibition of the licking reaction elicited by electrical stimulation of the tooth pulp in rabbits. Morphine (or saline) was applied either i. m. or intraventricularly for 3 weeks. Before and during the injection period the effects of test doses of morphine (10 mg/kg– 40 mg/kg i.m.) were checked at one week intervals. Controls: Stimulation threshold before injection of the test dose. The curves represent mean values and standard deviations of at least 8 animals.

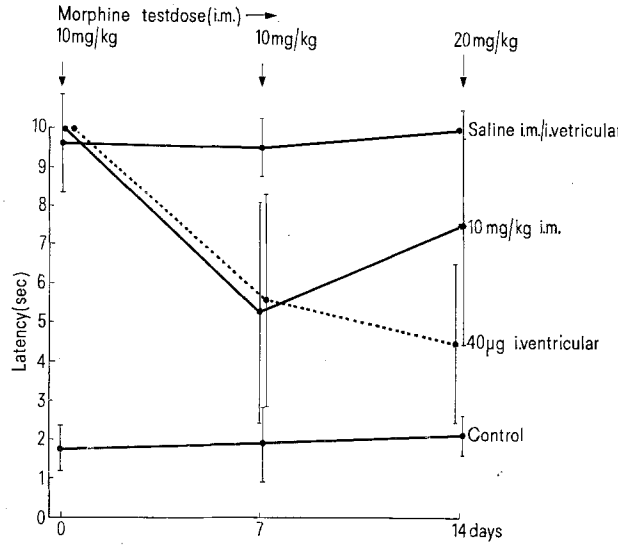

Fig. 2. Development of tolerance to the morphine-induced inhibition of the hindleg flexor reflex elicited by radiant heat to the paw in rabbits. Morphine (or saline) was applied either i.m. or intraventricularly for 2 weeks. Before and during the injection period the effects of test doses of morphine (10 mg/kg–20 mg/kg i.m.) were checked at one week intervals. Controls: Latency before injection of the test dose. The curves represent mean values and standard deviations of at least 6 animals.

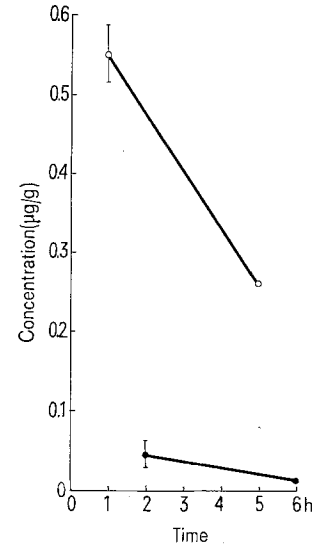

Fig. 3. Morphine concentrations in the lumbar spinal cord at different times after i.m. (20 mg/kg, ○) or intraventricular (80 µg, ●) injection of ^{14}C-morphine. Rabbits which had received morphine i.m. (20 mg/kg) or intraventricularly (80 µg) twice a day for a period of 14 days were used. Mean values and standard deviations of 3 experiments.

ment, the present results show that if such processes do occur, then they would take place in structures similar to those which are involved when morphine inhibits the tested reaction in the acute experiment. As also in the intraventricular series, the licking reaction was tested after systemic injection of the drug, one may conclude that the processes involved take place in structures easily reached when the drug is applied intraventricularly.

The present finding that inhibition of the hindleg flexor reflex also develops tolerance after intraventricular injection is of interest in view of the differentiation of spinal and supraspinal mechanisms involved in the inhibition of this reaction[3]. One might expect that spinal mechanisms are unmasked when the supraspinal mechanisms become tolerant. However, this does not seem to take place. In order to ascertain the above it is necessary to prove that, upon intraventricular injection of morphine insufficient amounts of the drug reach the lumbar spinal cord. For this purpose, ^{14}C-morphine was injected intraventricularly or i.m. in tolerant rabbits and its concentration in various segments of the spinal cord was determined by radioassay. The same radioassay technique was used as in previous experiments[3]. Figure 3 gives the mean morphine concentration in the lumbar spinal cord (L_1–L_6) at different times after i.m. and intraventricular injection. Upon intraventricular injection of 80 µg morphine, the concentration is far below the concentration reached after i.m. injection of 20 mg/kg (in both cases the equipotent doses of Figure 1 are doubled in order to increase radioactivity). However, a reliable comparison of these concentrations is only possible when the distribution within the spinal cord segments is not too different upon both injection modes. Previous investigations, concerning the permeation of morphine from the ventricular lumen into periventricular tissue, showed that the front of the permeating substance already reaches a depth of almost 2 mm 1 h after intraventricular injection[11]. Since the cord at lumbar level has a diameter in anterior/posterior direction of about 4 mm and the drug can enter the cord from all sides, one may suppose that no large gradients of morphine concentration are present even in the early hours after intraventricular morphine injection. Thus, the morphine concentrations given in Figure 3 for systemic and intraventricular morphine administration can be compared (see also VIGOURET et al.[3]).

In summing up the distribution studies, one may conclude that the morphine concentrations at the lumbar level after intraventricular injection are far below the level obtained after systemic injection of equipotent doses. Since the hindleg flexor reflex also developed tolerance upon intraventricular injection, it is evident that a spinal site of action of morphine is not unmasked when tolerance develops. This is a further indication that supraspinal structures exert powerful inhibition on nociceptive spinal reflexes.

Zusammenfassung. Die Entwicklung von Toleranz bei der Hemmung nociceptiver Reaktionen (Leckreaktion und Flexorreflex am Kaninchen) nach fortgesetzter intraventrikulärer und systemischer Morphininjektion wurde vergleichend untersucht. Beide Reaktionen entwickeln bei beiden Applikationsweisen des Morphins eine ähnliche Toleranz, obwohl im Fall des Flexorreflexes die Schaltstellen des Reflexbogens im Rückenmark nur von geringen Morphinkonzentrationen erreicht werden.

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