study of mechanisms which control each vessel. We suggest that the vascular system with the greatest amount of smooth muscle will give the greatest response to a stimulus, other things being equal, and thus responses and mechanisms should be easier to identify.

Based purely on morphological evidence, the guinea pig should be a good animal model for studying the mechanisms of control of the intrahepatic portal vein. Since the artery has little muscle in its wall, the portal vein must be more important for controlling inflow into the liver. The hepatic veins have little muscle except near the termination of the veins into the inferior vena cava and, thus, control of hepatic outflow must be poor.

The raccoon has a very interesting arrangement of muscle on the hepatic vein which must be important in controlling intrahepatic distribution or hepatic outflow.

The most interesting hepatic vessels seen by this author are the portal veins of the monitor lizard. These vessels have numerous remarkable doughnut-shaped sphincters which should be capable of intricate control of blood flow through the liver[12].

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0014-4754/85/020262-04$1.50 + 0.20/0


## Influence of hydrochlorothiazide on the pain threshold and on the antinociceptive activity of morphine, in rats[1]

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*Summary.* Hydrochlorothiazide, acutely injected in rats, has a weak analgesic activity per se and potentiates and prolongs the antinociceptive effect of morphine.

*Key words.* Rat; hydrochlorothiazide; pain threshold; antinociceptive activity; analgesic activity, morphine.

The involvement of $Na^{+2-4}$, as well as of other cations ($Ca^{++}$, $Mg^{++}$, $Mn^{++}$)[5,6], in the actions of narcotic analgesics is definitely established. Pert and Snyder[2] and Simon et al.[3] reported that $Na^+$ selectively affects the stereospecific binding of narcotic analgesics in mouse brain homogenates, an increase in $Na^+$ concentration decreasing the stereospecific binding of agonists and increasing the stereospecific binding of narcotic antagonists. In vivo, Lujan et al.[4] showed that large quantities of NaCl, given i.p. to mice, decrease the antinociceptive activity of morphine.

These findings prompted us to investigate the effects of increased natriuresis on the pain threshold and on the antinociceptive activity of morphine.

*Methods.* Male rats of a Wistar strain (Morini S.Polo d'Enza, Reggio Emilia), weighing 240–280 g, were randomly selected for treatment or for control experiments. Basal pain thresholds and the antinociceptive activity of morphine (morphine sulphate, Carlo Erba, Milan) were determined by the hot-plate test (constant platform temperature: 55 °C), with a cut-off time of 60 sec. The effect of morphine was calculated as a percentage of the maximum possible effect (MPE) according to the formula[7]: (TL − BL/60 − BL) × 100 (TL = test latency; BL = baseline latency; 60 = cut-off time of 60 sec).

1 h before the experiment, rats were randomly assigned to five groups and substances administered by i.p. injection as follows: groups 1, 2 and 3 – hydrochlorotiazide, 1, 5 and 10 mg/

Influence of hydrochlorothiazide on pain threshold and on morphine activity. Hot plate test (55 °C).

| Treatment* (i.p., 1 h before morphine) | Baseline reaction latency (sec ± SE) (immediately before morphine injection)* | % increase in reaction latency (MPE)** at the following times after morphine injection (5 mg/kg i.p.)* | | |
|---|---|---|---|---|
| | | 15 min | 30 min | 60 min |
| Saline (27) | 4.58 ± 0.31 (443.8 ± 15.01) | 10.99 ± 3.74 (437.25 ± 14.35) | 8.70 ± 1.48 (423.9 ± 16.18) | 4.99 ± 1.73 (435.6 ± 16.38) |
| Hydrochlorothiazide, 10 mg/kg (28) | 6.14 ± 0.82● (435.6 ± 15.41) | 25.59 ± 6.13● (396.0 ± 4.66)● | 44.14 ± 6.88●● (394.2 ± 6.18) | 45.80 ± 9.09●● (376.28 ± 4.18)● |
| Hydrochlorothiazide, 5 mg/kg (10) | 6.62 ± 0.81● (424.0 ± 9.80) | 38.17 ± 14.50● (418.0 ± 15.86) | 45.81 ± 16.32● (433.41 ± 17.6) | 24.04 ± 11.58 (399.3 ± 3.30) |
| Hydrochlorothiazide, 1 mg/kg (10) | 5.21 ± 0.53 (429.0 ± 12.34) | 17.05 ± 4.61 (402.6 ± 6.02) | 30.72 ± 9.95● (409.2 ± 6.6) | 23.92 ± 9.82● (396.0 ± 4.66) |
| Mannitol, 2 g/kg (10) | 5.99 ± 1.60 (417.45 ± 11.23) | 5.10 ± 1.55 (427.35 ± 12.16) | 9.91 ± 1.25 (427.35 ± 10.9) | 11.12 ± 4.43 (433.95 ± 8.25) |

* No. of rats in brackets. ** See methods for details. ★ Figures in brackets are serum sodium concentrations (mg/dl). Each value is the mean ± SE for 8 rats. ● $p < 0.05$; ●● $p < 0.001$ (compared with controls at the same times) (Student's t-test).

kg, respectively; group 4 – mannitol, 2 g/kg; group 5 – saline, 1 ml/kg. The same treatment schedule was used for serum sodium determinations, and at 0′, 15′, 30′ and 60′ after morphine injection, eight rats per dose were killed by decapitation, blood was collected, and sodium concentration in serum determined by flame spectrophotometry (Zeiss PMQ II; emission at 589 nm).

Significances were determined using Student's t-test. Variability is expressed as the SE of the mean.

*Results and discussion.* Hydrochlorothiazide, acutely injected at the doses of 5 and 10 mg/kg i.p., had a poor but significant analgesic effect per se; moreover, at all the doses tested, it greatly potentiated and prolonged the antinociceptive activity of morphine (table), this effect being longer-lasting with the highest dose. Mannitol, which at our dose level induces essentially a water diuresis, neither modified per se the pain threshold, nor affected the effect of morphine. Sodium concentration in serum was significantly reduced only by the highest dose of hydrochlorothiazide (table).

Several biochemical studies have examined the mechanism of the effect of sodium on opiate receptors[8]. The enhancement of antagonist binding by sodium appears to be elicited by an accelerated dissociation of the endogenous opiate molecules from the opiate receptor[9,10]. Opiate receptor binding involves distinct high-and low-affinity sites[11]. The reduction in agonist binding produced by sodium is mediated by an abolition of high-affinity agonist binding sites[8].

However, the present results do not seem to indicate a clear-cut relationship between the morphine-potentiating effect of hydrochlorothiazide and the reduction in serum sodium concentration.

In fact, under our conditions, sodium concentration was significantly reduced only by 10 mg/kg of hydrochlorothiazide, whereas morphine analgesia was also significantly potentiated and prolonged by 5 and 1 mg/kg of hydrochlorothiazide.

It is possible that serum sodium concentrations do not exactly reflect the contemporaneous situation in tissues, and that the morphine-potentiating effect of hydrochlorothiazide nevertheless depends on a reduced $Na^+$ availability at the opiate receptor level. However, our present data can neither definitely prove such a possibility, nor exclude that this effect of hydrochlorothiazide is independent of its natriuretic activity.

1 This work was supported in part by grants from Consiglio Nazionale delle Ricerche, and by Ministero della Pubblica Istruzione, Roma.

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0014-4754/85/020265-02$1.50 + 0.20/0


## Ocular instillation of naloxone increases intraocular pressure in morphine-addicted patients: A possible test for detecting misuse of morphine

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*Summary.* The effect of conjunctival instillation of naloxone on intraocular pressure has been examined in morphine-addicted patients as compared to non-addicted healthy volunteers. Morphine-addicted subjects showed a lower basal value of intraocular pressure as compared to the control volunteers. The instillation of naloxone caused a normalization of intraocular pressure to a level similar to that of control volunteers. This test seems to be a useful screening method for detecting morphine addiction.
*Key words.* Morphine addiction; naloxone; conjuctival instillation; intraocular pressure.

*Introduction.* Pupillary constriction is induced by local application of morphine in the rabbit[1] and human eye[2,3]. While the topical administration of the opiate antagonist naloxone is unable to antagonize miosis induced by an acute parenteral dose of morphine conjuctival instillation of naloxone reverses miosis induced by morphine[3]. Moreover, naloxone induces mydriasis in morphine-addicted patients after conjunctival instillation[4], and this test has been suggested as a possible screening method in detecting morphine dependence. Patients addicted to morphine or heroin exhibit an increased aqueous outflow associated with a decrease in intraocular pressure[5]. Furthermore, the existence of opioid peptides has recently been demonstrated in the ox eye[6].

The present experiments were aimed at investigating the effect of local administration of naloxone on intraocular pressure in patients addicted to morphine. The results of the present work suggest the use of this naloxone test in detecting morphine addiction.

*Methods.* The study was performed on 10 patients (eight males and two females) at the Provincial Hospital 'Garibaldi' in Catania, 18–29 years old, who had been abusing morphine for at least one year, 100–200 mg/day. Four nonaddicted healthy volunteers (two males and two females, 20–24 years old) were studied as controls. 4 h after the last i.v. morphine administration to addicted patients, basal values of intraocular pressure were measured on both eyes of addicted and control subjects with a Schiötz tonometer (Sbisà, Florence, Italy). 1 h later the naloxone test was performed by instilling two drops (0.1 ml) of 20% naloxone saline solution in the right eye of addicted and control subjects. Only saline solution was instilled in the left eye. Measurements of intraocular pressure, started 30 min after the instillation of the drug, were performed four