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Effects of morphine and clonidine on sulphobromophthalein disposition in mice

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Levels of sulphobromophthalein (BSP) in plasma and liver were elevated by the opiate, morphine, and by the α_2-adrenoceptor agonist, clonidine. Neither morphine, 1 mg kg^{-1}, nor clonidine, 0·01 mg kg^{-1}, affected BSP levels significantly. When given together at these doses, they caused BSP levels in plasma and liver to be raised. At 20 mg kg^{-1}, the effect of morphine on BSP levels was maximal, as was that of clonidine, 1·0 mg kg^{-1}. However, the effect of these drugs given together on plasma BSP exceeded the maximal effect of either alone. Yohimbine, an α_2-adrenoceptor antagonist, did not affect BSP levels, nor did the opiate antagonist, naloxone. Each of these antagonists reversed the hepatobiliary effects of its respective agonist, as shown by return of BSP levels to those of saline-treated mice. Yohimbine did not reverse morphine, nor did naloxone reverse clonidine. The additive effects of morphine and clonidine and the specificities of their respective antagonists strongly suggest the involvement of discrete receptors mediating their essentially identical hepatobiliary effects.

Several studies in the past decade have shown interactions between opiates and the adrenergic nervous system. Schultzberger et al (1978) and Wilson et al (1980) showed that opioid peptides are found in adrenergic nerve endings and in the adrenal medulla, which are both sites of catecholamine storage. In the locus coeruleus, both opiate and adrenergic nerve endings have been identified (Aghajanian 1982). Clonidine, an α_2-adrenergic agonist, has been shown to alleviate the clinical symptoms of opiate withdrawal (Gold et al 1978; Washton et al 1980). Furthermore, morphine has an effect on adrenergic transmission (Hughes et al 1975) and noradrenaline release in-vitro (Montel et al 1974).

Sulphobromophthalein (BSP) is an anionic dye which has been used extensively to evaluate hepatobiliary function. Upon intravenous administration, this dye is rapidly taken up by the liver, conjugated with glutathione and secreted into bile. Prolonged retention of BSP in plasma is regarded as evidence of hepatobiliary dysfunction, reflecting impairment of hepatic blood flow, parenchymal cell functions or biliary secretion and flow (Kaplowitz et al 1982). Although currently BSP disposition is infrequently studied in clinical settings, it is still widely used to investigate hepatobiliary function. We have recently shown that opiates and the α_2-adrenergic agonist, clonidine, have nearly identical effects on the hepatobiliary disposition of BSP (Hurwitz et al 1985; Ben-Zvi & Hurwitz 1985). Both elevate BSP levels in liver and plasma, while reducing amounts excreted in bile. We, therefore, chose to study interactions between clonidine and morphine and their specific antagonists on the retention of BSP in plasma and liver.

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Methods

Male Swiss Webster mice, 25–35 g were housed, 10 per cage, over crushed corn cob bedding at 23–25 °C. A 12 h dark–light cycle was followed, which was switched at 0600 and 1800 h. Purina rodent chow and water were freely available. Mice were originally obtained from Arther Sutter Co., Springfield, MO, and subsequently from Lab Supply Co., Indianapolis, IN. In each experiment, we report results from animals that had been delivered from a single supplier. Sulphobromophthalein sodium (BSP) was obtained from Hynson Westcott and Dunning, Baltimore, MD, and from Aldrich Chemical Co., Milwaukee, WI. Morphine sulphate was obtained from Eli Lilly, Indianapolis, IN; clonidine hydrochloride was from Boehringer Ingelheim, Ridgefield, CT; naloxone hydrochloride was from Endo Laboratories, Garden City, NY; and yohimbine hydrochloride was from Sigma Chemical Co., St Louis, MO, USA.

In a typical experiment, mice were injected subcutaneously with saline, morphine or clonidine or their antagonists 30 min before intravenous BSP. Thirty minutes after dye administration, blood was obtained from the orbital sinus and the animals were killed and their livers removed for dye determination. At these times, morphine and clonidine had their maximal effects on BSP disposition (Hurwitz et al 1985; Ben-Zvi & Hurwitz 1985). BSP was analysed in plasma spectrophotometrically at 580 nm after appropriate dilution of samples in 0·1 M sodium hydroxide. The dye content of

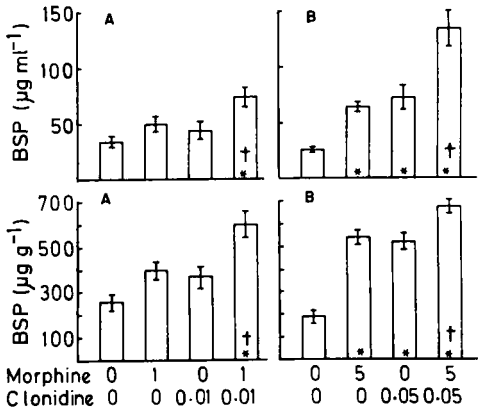

FIG. 1. Effects of morphine (upper Figs) and clonidine on BSP disposition. Morphine, clonidine or saline were injected s.c. 30 min before BSP, 125 mg kg^{-1} i.v. Blood was obtained 30 min later and the mice were then killed and livers removed. Morphine and clonidine doses in mg kg^{-1} are indicated below the figure. n = 12 in each group in experiment A and 15 in each group in experiment B. Data are means ± s.e.m. $*P < 0.05$ compared to saline control. $\dagger P < 0.05$ compared with either agonist alone.

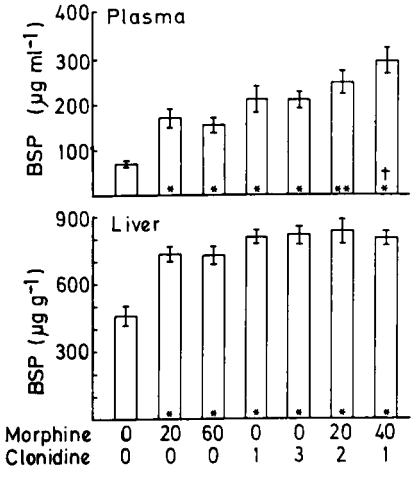

FIG. 2. Effects of additional morphine or clonidine to maximal doses of either agonist. Morphine, clonidine or saline were injected s.c. 30 min before BSP, 125 mg kg^{-1} i.v. Blood was obtained 30 min later and the mice were then killed and livers removed. Morphine and clonidine doses in mg kg^{-1} are indicated below the figure. n = 15–16 in each group. Data are means ± s.e.m. $**P < 0.05$ compared to morphine alone. $*P < 0.05$ compared with clonidine alone.

liver was determined at the same wavelength following methanol extraction, according to the method of Whelan & Combes (1971). Data were analysed for statistically significant differences by analysis of variance and the new Duncan's test (Steel & Torrie 1960).

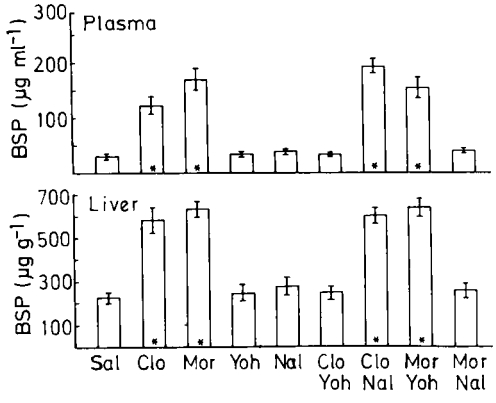

FIG. 3. Effects of opioid and adrenergic agonists and antagonists on BSP disposition. Mice were injected s.c. with saline, morphine sulphate, 20 mg kg^{-1}, clonidine hydrochloride, 0·2 mg kg^{-1}, naloxone, 1 mg kg^{-1}, or yohimbine, 2 mg kg^{-1}. Thirty min later, BSP was injected i.v. at 125 mg kg^{-1}. After another 30 min blood was obtained, the animals killed, and their livers removed. n = 8 in the saline-treated group and 12–13 in each of the other groups. Data are means ± s.e.m. $*P < 0.05$ compared with saline-treated controls.

Results

The combined administration of morphine and clonidine caused greater retention of BSP in plasma and liver than either drug given alone. When given together, doses of morphine and clonidine which alone had no significant effects on BSP disposition, caused plasma and liver dye levels to rise significantly (Fig. 1A). Higher doses of morphine or clonidine did raise plasma and liver levels of BSP (Fig. 1B). When these doses were combined, BSP levels rose even further. Doses of each drug in excess of those causing a maximal effect, i.e. morphine, 20 mg kg^{-1} or clonidine, 1·0 mg kg^{-1} had no additional effect on BSP levels. However, addition of clonidine to a maximally effective dose of morphine, or of morphine to clonidine, caused further elevation of plasma BSP, but not of hepatic dye retention (Fig. 2).

Yohimbine and naloxone, which are, respectively, adrenergic and opiate antagonists, had no significant effect on BSP disposition. Each of these antagonists reversed the elevation in plasma and liver BSP levels caused by its specific agonist, with naloxone reversing morphine, and yohimbine, clonidine (Fig. 3). Morphine raised plasma and liver BSP regardless of the presence or absence of yohimbine, and clonidine raised these levels despite co-administration of naloxone.

Discussion

Morphine and clonidine share many pharmacological properties. Clonidine has been used to control withdrawal from narcotics in addicted patients (Gold et al 1978; Washton et al 1980). In rodents, both clonidine and morphine have antinociceptive effects, and each reduces the ED50 of the other for suppression of

response to painful stimuli (Paalzow 1979). The α-adrenoceptor antagonist, phenoxybenzamine, suppressed the analgesic effect of morphine injected into the nucleus reticularis gigantocellularis (Kuraishi et al 1978). Yohimbine, an α_2-antagonist, blocked both morphine- and clonidine-induced analgesia but the opiate antagonist, naloxone, only reversed morphine-induced analgesia (Fielding et al 1980; Aceto & Harris 1980). α_2-Adrenoceptor agonists and opiates have both been shown to reduce levels of cyclic AMP by inhibiting adenylate cyclase (Clare et al 1984; Duggan & North 1983). Despite all these similarities, and other evidence for commonness of effects, the specific sites of action apparently differ. Thus, morphine analgesia was absent in pithed mice, while the antinociceptive effect of clonidine was essentially unchanged (Fielding et al 1980).

We had previously shown that morphine and clonidine have essentially identical effects on the disposition of the anionic dye, BSP, in rodents (Hurwitz et al 1985; Ben-Zvi & Hurwitz 1985). Both drugs reduced dye elimination into bile, while elevating its levels in plasma and hepatic tissue. These effects were maximal at 30–60 min after dye administration. The effects of morphine and clonidine remained after reversal of drug-induced hypothermia and when bile duct spasm was prevented by duct cannulation. Impaired BSP metabolism was also excluded as a possible cause of enhanced dye retention. Morphine or clonidine-induced changes in hepatic blood flow remain the most likely mechanisms for the acute effects of both drugs on liver function. It has not been established if these drugs affect the liver by central, spinal, or peripheral actions and whether presumed vascular changes are primarily systemic or splanchnic.

The hepatobiliary effects of morphine and clonidine thus appeared quite similar, as had been reported for their antinociceptive properties. In the experiments which we currently present, we set out to determine if receptors, regardless of anatomical distribution, were shared in the hepatobiliary effects of morphine and clonidine. We showed that addition of morphine to clonidine, and vice versa, elevated plasma BSP levels beyond maximal effect of either agonist given alone. This suggests alternate pathways for these drugs. Further evidence for separate sites was provided by the studies with antagonists. The α_2-adrenoceptor antagonist, yohimbine, had no effect on BSP disposition. It did reverse the effects of clonidine, while leaving morphine action unaltered. Opiate specificity for morphine was confirmed by its reversal by naloxone, which in turn, had no effect on the action of clonidine. These studies, showing additive effects and inhibitor specificity, strongly suggest that opioids and adrenoceptor agonists, which have very similar effects on hepatobiliary function, do so by acting on separate and discrete receptors.

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