after a s.c. injection of 21.2 μg ^{14}C-BCNU. The relative organ distribution following s.c. injection was in accord with that following topical application, suggesting that oral ingestion was not a factor in the results.

Activities were found in all tissues examined indicating facile percutaneous penetration. Interpretation of data for the gut is difficult due to the likelihood of fecal contamination. Exclusive of the gut highest values were found in the liver, kidney and lung. High values for liver and kidney in mice were also found by Wheeler et al.[4] and De Vita et al.[5] following i.p. injection. We noted a sharp drop in values for all viscera between 2 and 6 h after topical application as did Wheeler et al. following i.p. injection[4]. Activity in the brain again demonstrates the ability of BCNU or its products to cross the blood-brain barrier[5].

Mouse skin is thinner and more hirsute than human skin. Using in vitro chambers, Marzuli et al.[6] found mouse skin to be many fold more permeable than human skin. Extrapolation of the present data to man must take this enhanced permeability of mouse skin into consideration. Percutaneous penetration of BCNU in man is the subject of another study in progress by the authors.

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Analgesic effects of 3-carboxysalsolinol alone and in combination with morphine

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Summary. A biphasic dose-response pattern is generated by the isoquinoline, 3-carboxysalsolinol, in analgesia tests conducted in mice. Carbidopa pretreatment enhances this effect, as well as the morphine-induced analgesic increase by 3-carboxysalsolinol. Naloxone blockade of all of these responses suggests an interaction of the alcohol-based isoquinoline with central opiate receptors.

Ross and his colleagues have shown that morphine (M), ethanol (ETOH) and salsolinol (SAL) can diminish calcium levels in brain regions. This action can be suppressed by the narcotic antagonist naloxone (N)[2]. SAL is a tetrahydroisoquinoline (TIQ) derived from condensation of the primary metabolite of ETOH, acetaldehyde, with dopamine[3–5]. We have reported that SAL and the related 3-carboxysalsolinol (3cSAL), derived from acetaldehyde and L-DOPA prolong ETOH-induced narcosis in mice, with 3cSAL being more potent by far[5]. The suggested opiate-related action of SAL in the calcium experiments together with the enhanced ETOH-based narcosis observations have led us to examine 3cSAL for in vivo effects associated with opiates. Accordingly, 3cSAL was given to mice, alone and with M, and analgesia was assessed. Parallel studies were conducted with equimolar quantities of the 3cSAL precursor, L-DOPA, for comparison.

Materials and methods. Analgesia was measured by a modification of Haffner's tailclip method[6,7]. Male, Swiss-Webster mice (20–25 g) received i.p. injections (15 μl/g) of 3cSAL, L-DOPA or M (hydrochloride) or combinations of 3cSAL or L-DOPA and M. All drug solutions were prepared in acidic saline (1 drop of concentrated HCl per 5 ml of saline). The use of acidic saline assisted dissolution of the amino-acid compounds. Studies were performed on mice that also received carbidopa (CD), given orally (120 μM/kg) 1 h prior to injections of other substances. The actions of N (1 mg/kg) on drug-induced analgesia was determined by administering the narcotic antagonist i.p. just prior to injecting the other drugs. Analgesia tests were conducted 30 min after injections of M and/or the amino-acid compounds, or acidic saline. The results were analyzed statistically by determinations of differences between sample proportions[8].

Results and discussion. The levels of analgesia produced by 3cSAL, by 3cSAL after administration of CD and by L-DOPA are shown in figure 1. It is evident that 3cSAL produces a dose-related biphasic pattern of analgesia with the optimal level (54%) occurring in mice receiving a dose of 200 μM/kg. A similar pattern of analgesia was produced when 3cSAL was administered to animals pretreated with the peripheral inhibitor of L-amino-acid decarboxylase, carbidopa[9]. Inhibition of this enzyme potentiated the 3cSAL effect about 50fold. In the presence of CD, optimal analgesia (40%) was obtained with a 4 μM/kg dose of 3cSAL. CD on its own produced no evidence of analgesic activity.

L-DOPA afforded a low but increasing level of analgesic activity. A dose of 200 μM/kg of this amino acid gave a 30% level of analgesia which is significantly lower ($p < 0.03$) than that produced by the equimolar quantity of its isoquinoline analogue. Although a possible biphasic pattern of L-DOPA-induced analgesia was not pursued in this study it may exist as Major and Pleuvry have shown that a much higher dose of L-DOPA attenuates analgesia[10].

Morphine-induced analgesia is illustrated in figure 2. As expected, increased levels of analgesia occurred with higher doses. Combined treatments of M and 3cSAL

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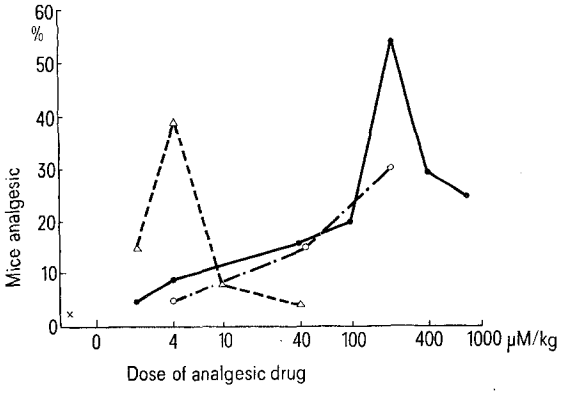

Fig. 1. Alteration of tail-clip analgesia in mice by 3-carboxysalsolinol, with or without carbidopa pretreatment, and by L-DOPA. At least 30 mice were used in each experiment. Analgesia testing was conducted 30 min after injections. The responses to 3cSAL (from 40 to 800 μM/kg) and to L-DOPA (40 and 200 μM/kg) are significantly different from the acidic saline control ($p < 0.05$). The response to 3cSAL (4 μM/kg) with CD pretreatment is significantly different from the non-pretreated control ($p < 0.015$). ×, Acidic saline control; ○–.–.– L-DOPA; ●——, 3-carboxysalsolinol; △– – –, 3-carboxysalsolinol with carbidopa (120 μM/kg) pretreatment.

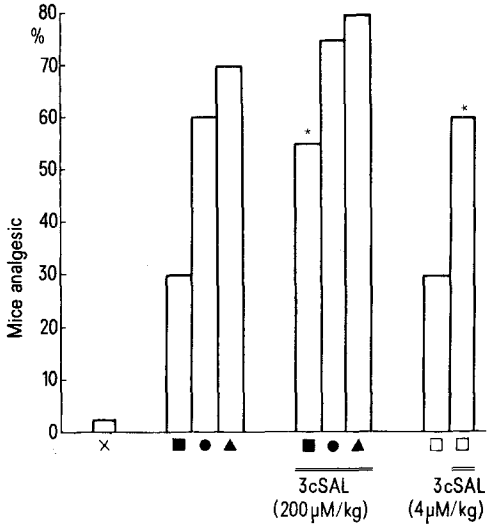

Fig. 2. Alteration of tail-clip analgesia in mice by morphine with 3-carboxysalsolinol co-treatment and carbidopa pretreatment. At least 30 mice were used in each experiment. The analgesia tests were conducted 30 min after the injections. An asterisk (*) indicates values significantly different from the 13 μM/kg morphine value ($p < 0.05$). ×, Acidic saline control; ■, morphine (13 μM/kg); ●, morphine (20 μM/kg); ▲, morphine (27 μM/kg); □, morphine (13 μM/kg) with carbidopa (120 μM/kg).

(200 μM/kg) produced enhanced levels of analgesia although significance was demonstrated with only the lowest M dose (13 μM/kg). CD did not alter analgesia associated with this dose of M. However, the combination of M (13 μM/kg) and 3cSAL (4 μM/kg) in CD-treated mice resulted in a significant increase in the level of analgesia. In contrast, L-DOPA (200 μM/kg) had no influence on analgesia produced by M. It was similarly unaltered in CD-treated mice receiving the low dose of M and L-DOPA (4 μM/kg).

Blumberg et al. first demonstrated the ability of N to counteract the actions of opiates[11]. In the present study, co-treatment with N abolished analgesia associated with the maximal responses to 3cSAL, 3cSAL with CD and L-DOPA (figure 1), and with M and combinations of M and other substances (figure 2).

The evidence presented here shows that 3cSAL, or its decarboxylated product SAL, can express an analgesic response that is antagonized by N. This suggests the possibility of an action on central opiate receptors[12]. Recent work has shown that SAL has affinity for the opiate receptors in the field-stimulated guinea-pig ileum[13] on which it acts as a weak agonist-antagonist. The biphasic analgesia pattern evinced by 3cSAL may represent a higher affinity of the isoquinoline for the agonist receptomer with increasing interaction with the antagonist configuration occurring at greater concentrations.

Co-treatments of 3cSAL (200 μM/kg) and M consistently increased the level of analgesia over that of M alone. The application of the conservative evaluative sample proportions method of analysis revealed that the accepted level of significance was achieved with only the lowest dose of M. Even so, this cannot be considered to reflect an additive M-3cSAL interaction as the increased level of analgesia is not significantly different from that produced by the 3cSAL alone. The possibility of such an interaction occurring is, however, suggested by the study in which 3cSAL (4 μM/kg) was combined with the low dose of M in CD-pretreated mice. In this case, the elevation of analgesic responses were significantly higher than those of M alone, M in decarboxylase inhibited animals and of the isoquinoline in the pretreated mice. This study indicates that 3cSAL, or SAL, can produce opiate-associated analgesia. The present in vivo results provide some endorsement for hypotheses that isoquinolines may unify ethanol and opiate dependencies[14,15].

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Effects of carmine and carminic acid on embryonic tissue cell cultures

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Summary. The biological reaction to carmine and carminic acid at cellular level on 'in vitro' cultures was tested and significant variables were controlled. Results suggested that proliferation and metabolism of these cultures were not affected by the 2 stains.

Recently[1,2] it was suggested that carmine induced damaging action with teratogenetic effects in mice. In the present work, the biological reactions at the cellular level on both hanging-drop and monolayered 'in vitro' tissue cultures were carefully studied. Cultures were from embryonic myocardium and tendons of chicken and several mammalian species. Carmine or carminic acid solutions were supplemented to the culture medium (plasma and