those mixtures or alcohols with a transition point ≤29 °C (the temperature of the experiment) exhibit a higher reactivity. Such an explanation may also account for the high reactivity seen with the two branched chain alcohols but unfortunately no transition point data for these alcohols exist in the literature.

In conclusion, it can be seen that the method of immersion calorimetry can be used quantitatively to assess the reactivity of the fatty alcohols with cetrimide. The measurements are relatively easy to perform and the results are consistent with the known theories of gel network formation.

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Locomotor activity and contracture of isolated ileum precipitated by naloxone following treatment of guinea-pigs with a single dose of morphine

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Guinea-pigs treated with a single dose of morphine, 15 mg kg^{-1} s.c., exhibited an increase in locomotor activity 2 h later on injection of naloxone, 4 mg kg^{-1} i.p. At the same time, contracture of ileal preparations isolated from morphine-treated guinea-pigs occurred on addition of naloxone 1 μM. Contracture of the ileum was inhibited by the tachykinin antagonist, spantide, and was therefore presumably mediated by a substance P-like agent. This study has established a useful model for the parallel investigation of central and enteric nervous system mechanisms of opiate dependence.

The guinea-pig ileum may be made dependent on opiates by pretreating the animal with morphine (Gintzler 1980) or by incubating the isolated ileum in-vitro with morphine (Collier et al 1981). Dependence may be induced following very brief, 2 min, exposure of ileum to met^5-enkephalin (Chahl 1983) and other opiates (Chahl, unpublished observations). The withdrawal response of ileum following 2 min exposure to met^5-enkephalin was revealed as a contracture following washout of met^5-enkephalin or addition of naloxone. Furthermore, a substance P (SP)-like agent apparently played a major role in the withdrawal contracture since it was inhibited by a tachykinin antagonist (Chahl 1983).

Since opiate dependence of isolated ileum occurred rapidly, signs of withdrawal should be apparent in animals following treatment with a single dose of morphine. The present study was undertaken to determine whether behavioural, as well as gastrointestinal, manifestations of withdrawal occurred in guinea-pigs treated with a single dose of morphine in-vivo, and also to determine whether the withdrawal contracture of the ileum following morphine in-vivo was similar to that following incubation with met^5-enkephalin in-vitro.

Methods

Adult guinea-pigs of either sex, 400–600 g, were used. They were treated subcutaneously with either morphine sulphate, 15 mg kg^{-1}, or an equivalent volume of saline 0·9% w/v (controls), and 2 h later both control and morphine-treated guinea-pigs were given naloxone hydrochloride, 4 mg kg^{-1}. Behavioural signs of withdrawal observed in morphine-treated guinea-pigs

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included increased locomotor activity, shivering, chewing, clawing at the cage floor and grooming. Preliminary experiments showed that locomotor activity was the most suitable behavioural withdrawal sign in guinea-pigs to quantify, particularly since guinea-pigs caged separately exhibited a low level of basal locomotor activity. Locomotor activity was therefore monitored in an activity cage with two electrical floor contacts at diagonal corners of the cage mounted between a double plastic floor. The cage design allowed only half or full circuits, but not fine movements by the guinea-pigs to be monitored. Cumulative numbers of counts were displayed on a digital counter and a signal corresponding to each count was simultaneously displayed on a chart recorder. Locomotor activity of each guinea-pig was monitored for 1 h before, and for 2 h following injection of morphine or saline, and for a further 1 h after naloxone injection.

For experiments on isolated ileum, guinea-pigs were killed by a blow to the head 2 h after injection of morphine or saline. A segment of distal ileum was removed and placed in a 2 mL organ bath under 1 g tension in Tyrode solution at 37 °C, gassed with oxygen.

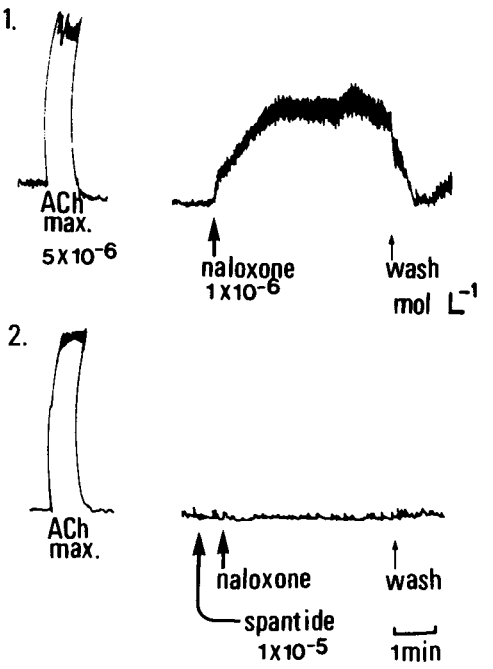

FIG. 2. Responses of ileum preparations from morphine-treated guinea-pigs. The traces show the responses to naloxone (1 μM) of ileum preparations from two different guinea-pigs treated with morphine sulphate 15 mg kg^{-1}. The upper trace shows the response in the absence, and the lower trace in the presence of the substance P antagonist, spantide 10 μM.

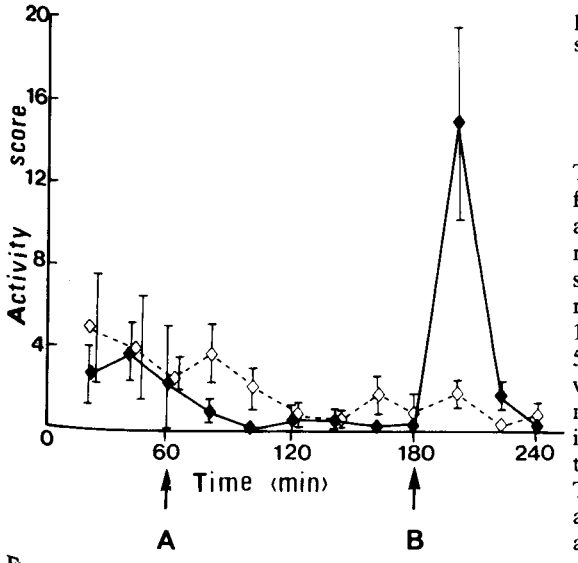

FIG. 1. Locomotor withdrawal responses of guinea-pigs. Points represent mean locomotor activity scores. Key: mean activity scores for control guinea-pigs given saline (at A) (◇), and for animals treated with morphine sulphate 15 mg kg^{-1} subcutaneously (at A) (◆). During the 20 min period after injection of naloxone hydrochloride, 4 mg kg^{-1} i.p. (at B), a significant increase in activity of animals treated with morphine occurred ($0{\cdot}05 > P > 0{\cdot}01$) but not in control animals. Bars are s.e. of means from 7 animals.

To avoid variable periods of washing out of morphine from the tissues, the following sequence of drug additions was adhered to strictly. The height of the maximum response of 5 μM acetylcholine (ACh) was standardized on the Grass polygraph chart. The recovery of sensitivity of the preparation was checked 15 min later by obtaining a response to ACh, 10 μM, and 5 min later naloxone, 1 μM was added to the bath and washed out after 5 min. Finally the sensitivity to SP, 2·5 nM, was tested 10 min later. Similar experiments using ilea from other animals were performed with either the tachykinin antagonist, spantide, (D-Arg^1,D-$Trp^{7,9}$,Leu^{11})-SP (Bachem), 10 μM, or atropine, 5 μM, added to the bath 30 s or 5 min, respectively, before addition of naloxone. Spantide was added 30 s before addition of naloxone since in a previous study this time was found to be adequate to inhibit markedly responses to SP (Chahl 1985).

Results

The mean activity scores for control and morphine-treated guinea-pigs are shown in Fig. 1. Locomotor activity decreased more rapidly in animals given mor-

phine compared with control animals but by 180 min both groups had low activity scores. Following injection of naloxone hydrochloride, 4 mg kg^{-1} i.p., there was a marked increase in activity score for the morphine-treated animals but no change in the activity of the control animals. The mean score for locomotor activity for the 20 min period following naloxone injection was significantly greater for morphine-treated guinea-pigs than for controls ($0{\cdot}05 > P > 0{\cdot}01$, Student's t-test). There was also a significant increase in activity of morphine-treated animals during the first 20 min period following naloxone injection compared with that during each of the three 20 min periods before morphine injection ($0{\cdot}05 > P > 0{\cdot}01$).

Addition of naloxone, 1 μM, to the isolated ileum from guinea-pigs pretreated 2 h previously with morphine produced marked contracture (Fig. 2), whereas addition of naloxone to ileal segments from control animals produced little or no contracture. The mean height of contracture of ilea from morphine-treated guinea-pigs (50 ± 7% of the ACh maximum, mean ± s.e., n = 5) was significantly greater than that from controls (13 ± 8%, n = 5) ($0{\cdot}01 > P > 0{\cdot}001$). The responses to SP and ACh were similar on preparations from morphine-treated and control guinea-pigs. Atropine, 5 μM, added 5 min before naloxone, did not significantly affect the height of the naloxone-induced contracture of ilea from morphine-treated guinea-pigs (44 ± 10%, n = 8), but spantide, 10 μM, abolished the response (0 ± 0%, n = 5) (significantly different from mean response in absence of spantide, $P < 0{\cdot}001$) (Fig. 2). Responses to SP were also abolished by spantide.

Discussion

As predicted from previous experiments on isolated ileum (Chahl 1983), dependence on opiates occurred very rapidly. Two hours after guinea-pigs were pretreated with a single dose of morphine, naloxone precipitated an increase in locomotor activity and other signs of central nervous system withdrawal as well as contracture of isolated ileum indicative of enteric nervous system withdrawal. Rapidly induced dependence on morphine, as measured by withdrawal precipitated by naloxone, has been observed previously in mice (Kosersky et al 1974) and in dorsal horn neurons where naloxone-precipitated firing occurred within 10 min following contact with morphine (Johnson & Duggan 1981).

It has been proposed that SP or a related substance is the primary mediator of the opiate withdrawal response of guinea-pig ileum, not only of the atropine-resistant component as has been suggested (Gintzler 1980; Tsou et al 1985), but also of the atropine-sensitive component (Chahl 1983). Therefore spantide was used in the present experiments since it is a relatively potent antagonist of the direct muscle action of SP on the ileum while retaining antagonist potency against the indirect (atropine-sensitive) response (Chahl 1985). The pharmacology of the ileal withdrawal contracture precipitated by naloxone following morphine treatment of guinea-pigs in-vivo was similar to that previously observed for met^5-enkephalin in-vitro (Chahl 1983), since it was abolished by a SP antagonist and therefore presumably mediated by SP or another tachykinin. However, atropine did not significantly inhibit the morphine withdrawal contracture and this agreed with previous findings for met^5-enkephalin withdrawal which was atropine-sensitive following 2 min contact with met^5-enkephalin, but became atropine-resistant following 32 min contact (Chahl 1983).

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