An unusual benzazocine elicits acetylcholine release in the isolated guinea pig ileum

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UM-1037 (3-allyl-1, 2, 3, 4, 5, 6-hexahydro-8-hydroxy-6-methyl-3-benzazocine), a benzazocine compound related structurally to the narcotic antagonists cyclazocine and the *N*-allyl analogue of cyclazocine, SKF-10,047, but lacking the 2,6-methano bridge, has been found to produce similar signs in normal rhesus monkeys to those characteristic of the narcotic abstinence syndrome[1]. Narcotic antagonists have been reported to produce contraction in the isolated guinea pig ileum[2,3]. However, only when the ileum has been continuously exposed to morphine or related drugs *in vitro* or has been isolated from animals made dependent on a narcotic, does it contract when an antagonist such as naloxone is added to the perfusion medium[4]. Thus, this preparation has been used as an *in vitro* model of the narcotic abstinence syndrome, and the contraction might be considered an 'abstinence sign'. In the present study, UM-1037 was found to produce a contraction of the guinea pig ileum isolated from normal animals similar to the contraction produced by naloxone in preparations exposed to morphine or related drugs. UM-1037, like naloxone, seems to act by releasing acetylcholine (ACh), because its actions were antagonised by atropine and tetrodotoxin. However, morphine, which is necessary for the naloxone-induced contraction, reversed the effect of UM-1037 on the ileum. Responses to UM-1037 were not altered in the presence of naloxone. Thus, the release of ACh which is produced by this unusual benzazocine in the ileum does not seem to be mediated by the opiate receptor.

The isolated guinea pig ileum was prepared as described by Paton[5]. Segments of ileum were suspended in a Krebs physiological buffer at 37 °C, saturated with 95% O_2–5% CO_2. The buffer contained NaCl, 118 mM; KCl, 4.75 mM; $CaCl_2.2H_2O$, 2.54 mM; $MgSO_4.7H_2O$, 1.19 mM; KH_2PO_4, 1.19 mM; glucose, 11 mM; $NaHCO_3$, 25 mM; hexamethonium Br, 0.07 mM; pyrillamine maleate, 0.125 μM. Resting tension on the tissue was 0.5 g. Tissues were equilibrated for 30–40 min with washes every 10 min. After the equilibration period, ACh, 5×10^{-6} M, was added to the bath to determine the maximum contraction of the tissue. Ten minutes after washing the ACh from the organ baths, cumulative concentration–response relationships were determined for UM-1037 by increasing the concentration of the drug by three-fold increments until a maximum response was obtained. After washout of UM-1037, ACh, 5×10^{-6} M, was added once again to determine whether the contractility of the preparation had changed.

Addition of UM-1037 to the organ bath produced a contraction of the isolated ileum (Figs 1, 2). The maximum contraction produced by UM-1037 was 53.5±8.1% of the contraction produced by ACh, 5×10^{-6} M, and ED_{50} for UM-1037 was $5.33\pm0.97\times10^{-6}$ M ($n = 8$). The effect produced by UM-1037 in the isolated ileum not exposed to morphine-like drugs resembles that produced by naloxone in preparations exposed to such drugs[2].

As the contraction produced by naloxone is inhibited by atropine, 10^{-7} M, and tetrodotoxin, 2×10^{-7} M, it is thought to be caused by a release of ACh from postganglionic cholinergic nerve terminals in the myenteric plexus[6]. To determine whether the contraction produced by UM-1037 was due to a similar mechanism, we investigated the effects of atropine and tetrodotoxin on this response. Atropine, 10^{-8} M or 10^{-7} M, added to the organ bath 20 min before UM-1037, antagonised the effects

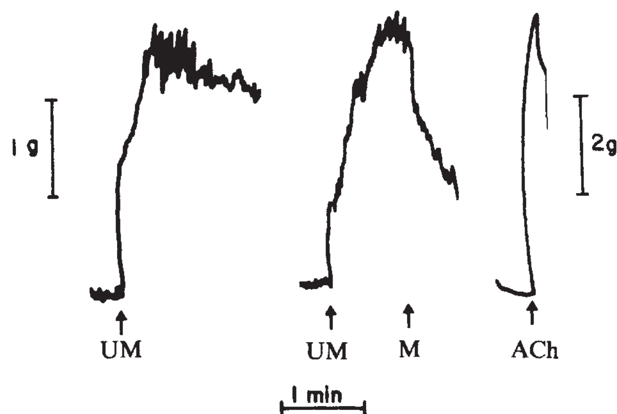

Fig. 1 Effects of UM-1037 on the isolated guinea pig ileum and antagonism by morphine. UM-1037, 3×10^{-5} M, was added to the organ bath at the time indicated by arrow UM. Morphine. 10^{-7} M, was added at arrow M. After washing the preparation, ACh, 5×10^{-6} M, a maximally effective concentration, was added to the organ bath at arrow ACh.

of UM-1037 (Fig. 2). In the presence of atropine, the concentration–response curve for UM-1037 was shifted to the right, and the maximum response was decreased. Tetrodotoxin, 2×10^{-7} M, also blocked the response of the ileum to UM-1037 when added 10 min before UM-1037 (Fig. 2). Although this concentration of tetrodotoxin inhibited the electrically induced contraction of the ileum, it did not affect the response to ACh. The interactions with atropine and tetrodotoxin suggest that UM-1037, like naloxone, produces a contraction of the tissue by releasing a limited store of ACh from cholinergic neurones; this in turn acts on the muscarinic receptors of the intestinal muscle.

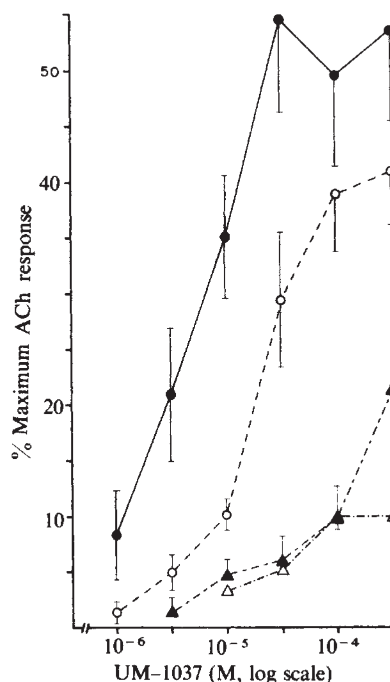

Fig. 2 Antagonism of UM-1037 by atropine and tetrodotoxin. Ordinate, per cent maximum contraction produced by ACh, 5×10^{-6} M. Abscissa, molar concentration. Concentration–response curves for UM-1037 in the guinea pig ileum are shown: eight control ilea (●——●); five ilea in the presence of atropine, 10^{-8} M, added to the bath 15 min before UM-1037 (○---○); five ilea in the presence of atropine, 10^{-7} M, added to the bath 15 min before UM-1037 (▲---▲); three ilea in the presence of tetrodotoxin, 2×10^{-7} M, added to the bath 10 min before UM-1037 (△—·—△). Mean values are given; vertical lines represent s.e.m.

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Morhpine-like agents inhibit the release of ACh from postganglionic cholinergic nerve terminals in the myenteric plexus of the guinea pig ileum[7,8]. This ability to inhibit ACh release is correlated with the ability to bind with specific receptors in the ileum[9]. To determine whether UM-1037 acts at specific opioid receptors, the interaction between UM-1037 and morphine was studied. Morphine, 10^{-7} M, reversed the contraction produced by a maximally effective concentration of UM-1037 (Fig. 1), and when added to the organ bath before UM-1037, it caused a parallel shift to the right in the UM-1037 concentration–response curve (Fig. 3). To ascertain whether muscarinic receptors in the ileum were affected by this concentration of morphine, the effect of carbachol was studied in the presence and absence of morphine. The ED_{50} values for carbachol alone, and when morphine, 10^{-7} M, had been added 5 min previously, were $1.15 \pm 0.09 \times 10^{-7}$ M ($n = 4$) and $1.37 \pm 0.38 \times 10^{-7}$ M ($n = 6$), respectively. Therefore, morphine reverses the effect of UM-1037 at a concentration which does not block muscarinic receptors. The antagonism of the effect of UM-1037 by morphine as well as by tetrodotoxin and atropine, further indicates that the contraction is due to release of ACh.

The interaction between morphine and UM-1037 may be due to competition at the same receptor site or to opposing effects mediated by interactions of the drugs at different receptor sites. Naloxone, a competitive narcotic antagonist, was used to elucidate this interaction further. Concentration–response relationships for UM-1037, determined 5 min after the addition of naloxone, 10^{-7} M, to the bath, did not differ from those for UM-1037 alone (Fig. 3). This suggests that naloxone and UM-1037 occupy different receptor sites. Naloxone, when added 5 min before morphine, 10^{-7} M, prevented the reversal of the effect of UM-1037 by morphine (Fig. 3). Because naloxone competes with morphine for opiate receptor-binding sites, it is probable that the antagonism of UM-1037 by morphine is mediated through the interaction of morphine with the opiate receptor to decrease ACh release in the guinea pig ileum. These results suggest that the interaction between UM-1037 and morphine does not occur by competition at a common receptor.

To determine whether UM-1037 is a narcotic antagonist, the effect of morphine was studied in the presence and absence of UM-1037. The preparation was coaxially stimulated at 0.1 Hz and supramaximal voltage for 1 h. Then cumulative concentration–response relationships were determined for morphine alone and repeated on other segments of tissue when UM-1037, 10^{-6} M, had been added 5 min previously. UM-1037 decreased the maximum inhibition produced by morphine (24.4% decrease), but did not produce a parallel shift to the right of the morphine concentration–response curve. The ED_{50} values for morphine in the presence and absence of UM-1037, 10^{-6} M, were $2.97 \pm 0.55 \times 10^{-8}$ M ($n = 5$) and $2.33 \pm 0.97 \times 10^{-8}$ M ($n = 5$), respectively. These results are consistent with the concept that morphine and UM-1037 do not compete for common receptors, and that UM-1037 decreases the maximum effect of morphine in the guinea pig ileum through an interaction with another site.

Prior exposure to a narcotic is a requirement for antagonist-precipitated abstinence in animals as well as for the antagonist-precipitated contraction of the isolated guinea pig ileum. The mechanism of narcotic antagonist-precipitated abstinence is generally thought to involve displacement of the narcotic from receptor sites.

In this study, the effects of UM-1037 on the isolated guinea pig ileum were studied. In this preparation, as in the monkey, a sign considered to be characteristic of narcotic abstinence was produced in the absence of narcotic exposure. The effects produced by this drug in the isolated guinea pig ileum resemble those produced by naloxone in preparations exposed to morphine in that release of acetylcholine mediates the contraction of the tissue in both cases. Unlike the naloxone-precipitated abstinence sign in the ileum, the effect produced by UM-1037 is antagonised by morphine.

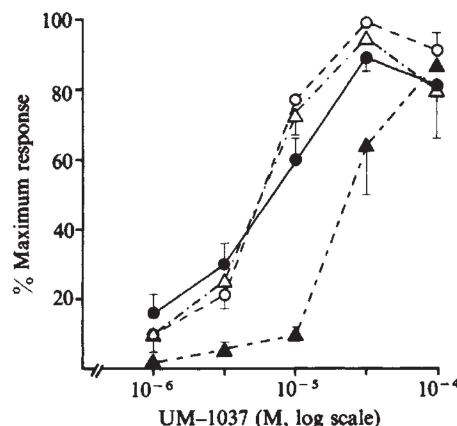

Fig. 3 Interaction between UM-1037, morphine and naloxone on the isolated guinea pig ileum. Ordinate, per cent maximum contraction. Abscissa, molar concentration. Concentration–response curves for UM-1037 in the guinea pig ileum are shown: eight control ilea (●——●); four ilea in the presence of morphine, 10^{-7} M, added to the bath 5 min before UM-1037 (▲---▲); three ilea in the presence of naloxone, 10^{-7} M, added to the bath 5 min before UM-1037 (○---○); four ilea in the presence of both naloxone, 10^{-7} M, added to the bath 10 min before UM-1037 and morphine, 10^{-7} M, added to the bath 5 min after naloxone and 5 min before UM-1037 (△—·—△). Mean values are given; vertical lines represent s.e.m.

A syndrome resembling the narcotic abstinence syndrome has been described in normal rats after the administration of phosphodiesterase inhibitors[11]. This 'quasi-abstinence syndrome' is antagonised by narcotics and potentiated by naloxone. However, UM-1037 does not act like a phosphodiesterase inhibitor. Although morphine antagonises the effect of UM-1037 in the isolated guinea-pig ileum, naloxone does not alter the response to UM-1037. Furthermore, theophylline, a methylxanthine which is a phosphodiesterase inhibitor, does not produce a contraction of the isolated guinea pig ileum, although the methylxanthines do enhance the electrically induced contraction of the ileum[12]. At high concentrations theophylline inhibits the twitch of the stimulated preparation[12]. UM-1037 produces a contraction of the isolated ileum and does not inhibit the twitch at any concentration. Therefore, UM-1037 and phosphodiesterase inhibitors do not seem to share a common mechanism of action in this preparation.

Like UM-1037, nicotine and 5-hydroxytryptamine cause a release of ACh from cholinergic neurones in the isolated guinea pig ileum[12]. Preliminary studies indicate that the actions of UM-1037 on this preparation are not mediated by either nicotinic or tryptaminergic receptors[13,14].

Although the present study suggests that the precipitation of the abstinence syndrome by narcotic antagonists in morphine-dependent ilea and the abstinence-like syndrome produced by UM-1037 in non-dependent ilea involve different receptor sites, it is possible that common mediators or neural pathways are involved in both drug effects. UM-1037 and related compounds may be important in elucidating the mechanism underlying the narcotic abstinence syndrome.

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Curare has a voltage-dependent blocking action on the glutamate synapse

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The skeletal muscle of members of Orthoptera and Diptera receives an innervation which is probably glutaminergic[1–3]. Recent study of the ventral muscle fibres in the larvae of the beetle, *Tenebrio molitor*, has revealed that the transmitter action can be mimicked by the iontophoretic application of L-glutamate to the junctional sites at which the extracellular excitatory postsynaptic potentials (e.p.s.ps) could be recorded (D.Y. and H.W., unpublished observation). Contrary to the evidence favouring glutamate as a transmitter of junctional excitation in insects, some investigators have found that curare (+)tubocrarine, TC), a classic acetylcholine (ACh) antagonist, suppresses the neurally evoked muscle potentials in the fly *Sarcophaga*[4,5], and *Tenebrio*[6]. Here, we have analysed the action of curare on the neuromuscular junction of *Tenebrio* larvae and found that curare blocked the glutaminergic transmission by antagonising the transmitter at the postsynaptic site.

The dissection used has been described previously[7]. The ventral muscle fibres were penetrated by two microelectrodes, one for recording and another for passing current, filled with 3 M KCl and 2 M K citrate, respectively. When necessary, the muscle fibres were voltage clamped using a two-microelectrode method[8]. For focal extracellular recording, electrodes were filled with 3 M NaCl. Although the signals recorded extracellularly could be resolved into single traces on the oscilloscope, a minicomputer (Sanei signal processor 7T07A) was used to obtain a signal averaging so as to improve the signal-to-noise ratio. The iontophoretic electrodes were filled with 1 M solution of monosodium L-glutamate (*p*H 8.0). The e.p.s.ps were evoked by stimulating the innervating nerve close to the segmental ganglion. All experiments were carried out in a low Ca^{2+} saline solution to any minimise movement artefacts accompanying an action potential. The standard bathing saline solution had the following composition (mM): NaCl, 70; KCl, 30; $CaCl_2$, 0.8; $MgCl_2$, 14.2; glucose, 445; HEPES, 5. The *p*H of the solution was adjusted to 7.2 with NaOH.

Curare, when applied to the bathing solution at concentrations higher than 5×10^{-4} M, reversibly depressed the e.p.s.p. amplitude without affecting either the input membrane resistance or the resting potential of the muscle fibre. Simultaneous focal recordings have revealed that the postsynaptic current was substantially depressed but that the presynaptic impulse and the synaptic delay remained essentially unchanged (Fig. 1). The quantum content estimated by the failure method[9] was unaffected by the application of curare (Table 1). These facts indicated that the site of action of curare was on the postsynaptic membrane. The observations do not necessarily mean that the presynaptic terminal of *Tenebrio* neuromuscular junction lacks

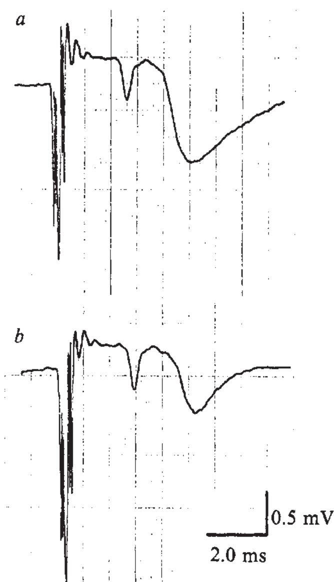
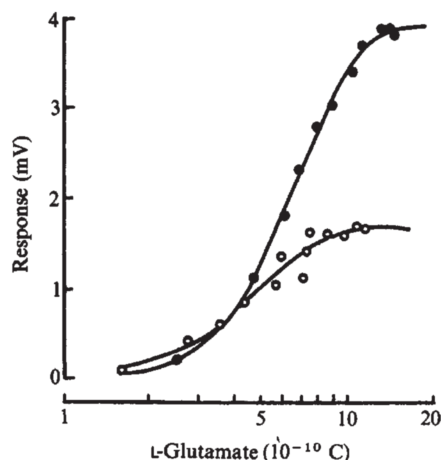


Fig. 1 The nerve terminal action potential and the e.p.s.p. recorded extracellularly from the neuromuscular junction. Records were taken before (*a*) and 5 min after (*b*) bath perfusion of 7.5×10^{-4} M curare. Signals were averaged over 99 trials.

the ACh receptors reported to be present on the locust junction[10], because curare failed to affect transmitter release even in the locust system unless it was applied simultaneously with ACh[10,11].

To characterise the curare-induced suppression further, we examined the action of curare on the glutamate potential. We found that the amplitude of the glutamate potential decreased and the time to peak was markedly prolonged after treatment with 5×10^{-4} M curare. One possible explanation for the prolonged glutamate potentials in TC solution is that the L-glutamate molecules ejected must diffuse over a greater distance to reach the sensitive receptors than would be required in normal conditions because some accessible receptors which are distributed on the fibre surface may become unavailable through the action of TC. Both effects of curare on the glutamate potential were fully reversible. Curare also produced a reduction in the apparent maximum of the dose–response curve for glutamate-induced depolarisation (Fig. 2). The non-competitive

Fig. 2 Effect of curare on the dose–response curve to glutamate recorded from a single mealworm muscle fibre: Results are of glutamate-evoked membrane depolarisation measured in control solution (●) and in 7.5×10^{-4} M curare (○).



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