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Evidence that the kappa agonist U50488H has non-opioid actions

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Abstract—The antagonism of the antinociceptive effects of various κ-opioid agonists has been studied in the mouse abdominal constriction test. Naloxone produced a much smaller degree of antagonism of U50488H than it did of two other κ-agonists, U69593 and tifluadom. The κ-selective antagonist, norbinaltorphimine, also failed to shift the dose-response curve to U50488H in this test, despite producing considerable antagonism of the U50488H effect in the rotarod test and of U69593 in both experimental situations. These results are suggestive of a non-opioid component to the action of U50488H in the abdominal constriction test. At high concentrations, U50488H, but not U69593, also showed non-opioid effects in reducing contractile activity in the field-stimulated isolated guinea-pig ileum, as demonstrated by the profile of antagonism seen with β-chlornaltrexamine and naloxone. These results suggest that U69593, rather than U50488H, may be the κ-agonist of choice to use, particularly in in-vivo experiments.

It is now well documented that there are subclasses of opioid receptors (Lord et al 1977; Martin et al 1976). This concept is of particular interest as it suggests the possibility of separating the desirable analgesic properties of opioid drugs from the unwanted side-effects like abuse potential and respiratory depression. In this respect, particular attention has been paid to the κ-receptor, for which a number of highly selective agonists are now available. Of particular note are the Upjohn compounds U50488H (Von Voigtlander et al 1983) and U69593 (Lahti et al 1985).

In the present experiments, we confirm that U50488H shows good selectivity for opioid receptors in-vitro, although at higher concentrations it does appear to have non-opioid actions. However, in the mouse abdominal constriction test in-vivo, U50488H, unlike U69593, appears to show very poor selectivity for opioid receptors.

Materials and Methods

In-vivo testing. Male, albino CRH mice (17–25 g) were used. Detailed methodology for the mouse acetylcholine-induced abdominal constriction test for the measurement of antinociceptive activity and the rotarod test for measurement of motor incapacitation are given in Hayes et al (1987). Opioid drugs were administered 20 min before testing. The non-selective opioid antagonist, naloxone, was co-administered subcutaneously with the opioid agonist. The κ-antagonist, norbinaltorphimine (norBNI; Birch et al 1987; Portoghese et al 1987) was administered i.v. 90 min before testing. All drugs were colour coded so that the operators were unaware of which treatment the animals were receiving. Individual tests were carried out using dose-groups of six animals and data were accumulated from two or three individual tests carried out on different days such that the final dose-groups comprised 12 or 18 animals. Dose-ratios (and 95% confidence limits) were calculated by the method of Finney (1964).

In-vitro testing. Segments of guinea-pig ileum were set up and electrically stimulated as described in Sheehan et al (1986), except that the calcium concentration of the Krebs was 1·25 mM instead of 2·6 mM. Cumulative agonist concentration-response curves were constructed and then the irreversible opioid antagonist β-chlornaltrexamine (β-CNA; Portoghese et al 1978), 10^{-7} M, was incubated with the tissues for 30 min. The tissues were then extensively washed for 1 h until the twitch height stabilized. The agonist concentration-response curve was then repeated. The tissues were then superfused with naloxone, 3×10^{-7} M, for 30 min, before redetermination of the agonist concentration-response curve.

Drugs. Drugs used were as follows: acetylcholine hydrochloride (Sigma), U50488H (*trans*-(±)-3,4-dichloro-*N*-methyl-*N*[2-(1-pyrrolidinyl)cyclohexyl]benzeacetamide methane sulphonate (Upjohn), U69593 (5α,7α,8β-(−)-*N*-methyl-*N*-(7-(1-pyrrolidinyl)-1-oxaspiro (4,5) dec-8-yl) benzeneacetamide (Upjohn),

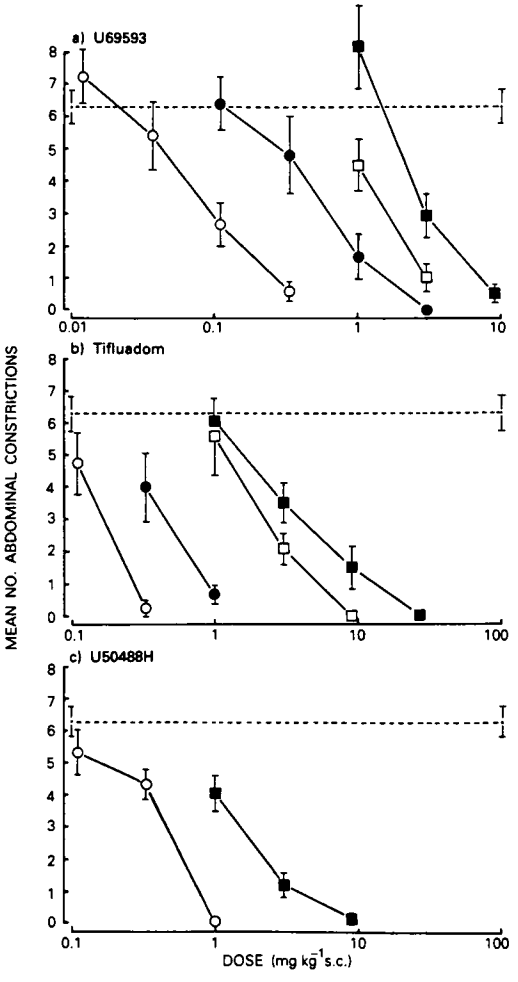

FIG 1. Naloxone reversibility of the antinociceptive effects of U69593, tifluadom and U50488H in the mouse abdominal constriction test. Values are means ± s.e. ($n > 12$). The dotted lines represent the values for vehicle treated controls. ○ represents opioid agonist alone ● in the presence of naloxone, 0·67 mg kg^{-1} s.c., □ in the presence of 2 mg kg^{-1} s.c. naloxone, ■ in the presence of 6 mg kg^{-1} s.c. naloxone.

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tifluadom hydrochloride (Kali-Chemi), β-CNA and norBNI, both hydrochlorides (synthesized by Dr. C. Meerholz and Dr. A. McElroy respectively, Chemical Research Department, Glaxo, Ware), naloxone hydrochloride (Sterling-Winthrop).

Results

In-vivo experiments. The κ-agonists U69593, tifluadom and U50488H all produced dose-related antinociceptive effects in the mouse abdominal constriction test (Fig. 1). Naloxone, 0·67 to 6 mg kg^{-1} s.c., produced dose-related antagonism of the antinociceptive effects of U69593 and tifluadom; however, naloxone produced a much smaller degree of antagonism of U50488H in this test (Fig. 1). Thus, naloxone 6 mg kg^{-1} s.c., produced shifts of U69593, tifluadom and U50488H of 40·2 (confidence limits 24·1–69·3), 25·1 (14·5–46·2) and 4·6 (2·9–6·8), respectively.

The κ-selective antagonist norBNI, 20mg kg^{-1} i.v., produced a large rightwards shift of the dose-response curve to U69593 in the abdominal constriction test, but no significant antagonism of U50488H. In the same experiments, the dose-response curves to both drugs in the rotarod test showed a considerable rightwards shift in mice pretreated with norBNI (Fig. 2).

In-vitro experiments. Both U69593 and U50488H produced concentration-related depressions of the twitch response in the field-stimulated guinea-pig ileum, with IC50 values of $3 \pm 0{\cdot}4$ nM and 4 ± 1 nM, respectively. Following treatment with the irreversible opioid antagonist β-CNA, 10^{-7} M for 30 min, the concentration-response curve to U69593 was shifted to the right and there was a decrease in the maximum effect (Fig. 3a). Subsequent treatment with naloxone caused a further parallel shift in the dose-response curve to U69593, yielding a pA_2 value of $7{\cdot}5 \pm 0{\cdot}2$ calculated using the Gaddum equation. Treatment with β-CNA also produced a rightwards shift in the concentration-response curve to U50488H in the guinea-pig ileum; however, after β-CNA treatment, the dose-response curve to U50488H appeared biphasic, with only the lower part of the curve being naloxone-sensitive (Fig. 3b). After treatment with a higher concentration of β-CNA, 10^{-6} M, the dose-response curve to U50488H was not shifted at all by naloxone, 3×10^{-7} M (data not shown).

U50488H inhibited contractile responses produced by exogenous acetylcholine, 10^{-7} M, and histamine, 10^{-7} M, with IC50 values of $20 \pm 0{\cdot}2$ μM and $7{\cdot}2 \pm 1{\cdot}3$ μM, respectively. U69593, $10^{-6} - 10^{-4}$ M, produced no inhibition of contractions produced by acetylcholine in the guinea-pig ileum.

Discussion

Naloxone produced a much smaller degree of antagonism of U50488H in the mouse abdominal constriction test than it did of the other κ-agonists, U69593 and tifluadom. The κ-selective antagonist norBNI also failed to shift the dose-response curve to U50488H in this test, despite producing considerable antagonism of the U50488H effect in the rotarod test and of U69593 in both experimental situations. The lack of antagonism of U50488H is unlikely to be because testing was carried out at the wrong time, as pilot experiments had shown that 20 min is around the time of peak effect of all 3 κ-agonists. Also, this seems unlikely to explain the differential antagonism of U50488H by norBNI in the abdominal constriction and rotarod tests. Overall, the results are suggestive of a non-opioid component to the action of U50488H in the abdominal constriction test. Von Voigtlander et al (1983) reported large shifts by naloxone of the dose-response curve to U50488H in the mouse tail-flick test, so there does not appear to be a non-opioid component to the action of U50488H in that test.

The profile of antagonism of U50488H in guinea-pig ileum is also suggestive of non-opioid activity at high concentrations, again an effect which was not apparent with U69593. However, the degree of selectivity seen with U50488H in guinea-pig ileum was clearly much better than that seen in the abdominal constriction test. The demonstration that U50488H reduced contractile responses to exogenous acetylcholine in guinea-pig ileum suggests that this underlies the non-opioid activity seen in this tissue. It is interesting to note that U50488H also decreased contractions to histamine in guinea-pig ileum (see Results) and,

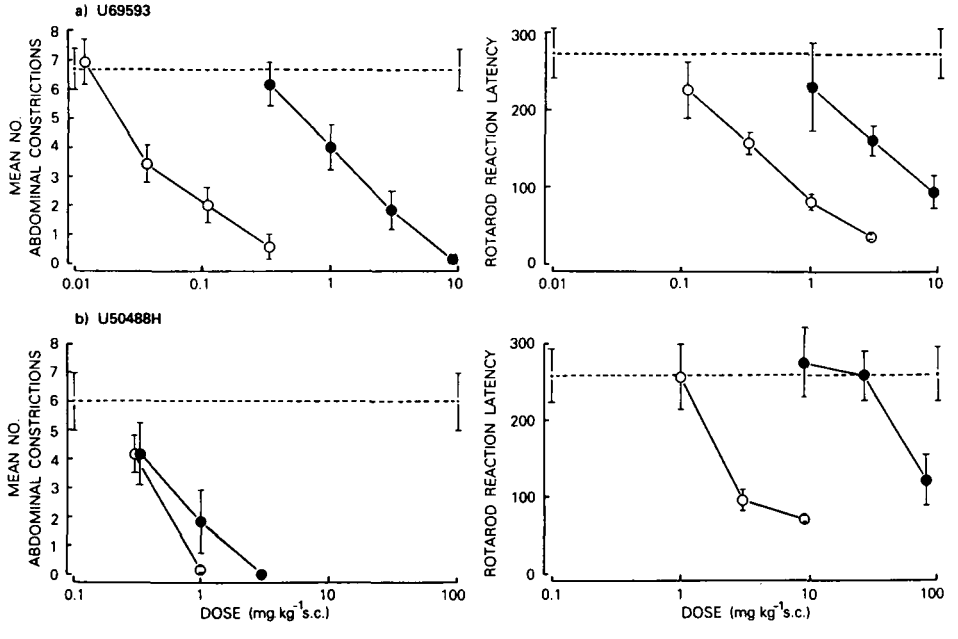

FIG 2. Antagonism by norBNI of the effects of U69593 and U50488H in the mouse abdominal constriction and rotarod tests. Values are means ± s.e. (n > 6). The dotted lines represent the values for vehicle treated controls. ○ represents opioid agonist alone, ● in the presence of norBNI, 20mg kg^{-1} i.v.

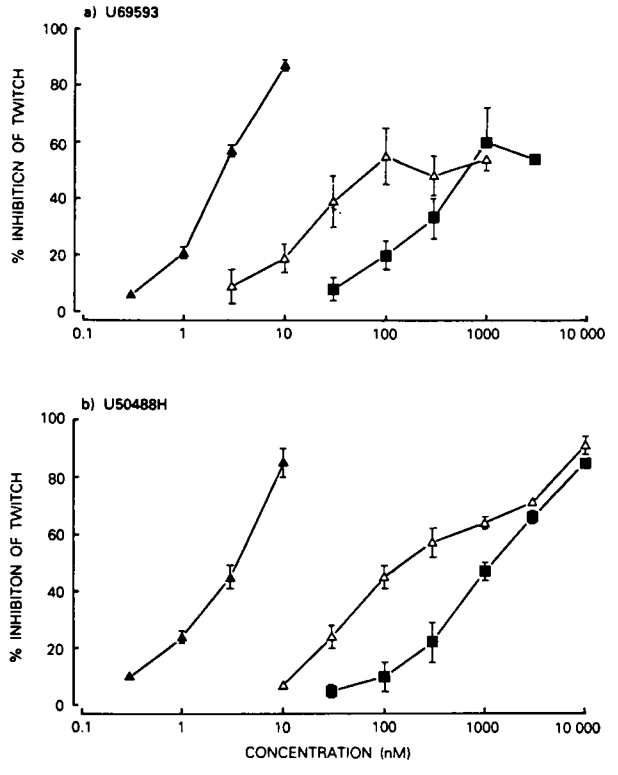

FIG 3. Inhibition of contractile responses produced by κ-agonists in the field-stimulated isolated guinea-pig ileum. Values are means ± s.e. (n > 4). ▲ represents responses to U69593 or U50488H alone Δ after treatment with βCNA, 10^{-7} M for 30 min ■ after treatment with βCNA and naloxone, 3×10^{-7} M.

at 10^{-4} M, decreased the contraction produced by phenylephrine, 10^{-5} M, in rat vas deferens (unpublished observations). This suggests that U50488H has a non-specific effect to reduce smooth muscle contractile responses. Kappa agonists are known to block voltage-sensitive calcium channels in dorsal root ganglion cells (Werz & Macdonald 1984) and one might speculate that, at high concentrations, U50488H also affects calcium channels in smooth muscle. It is impossible to say at the present time how this might relate to the effect seen in the abdominal constriction test.

In summary, the κ-agonist U50488H clearly has non-opioid actions, particularly in certain in-vivo situations. This suggests that U69593 is superior to U50488H for use as a selective κ-agonist in pharmacological experiments.

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