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Short communication

Apparent efficacy of κ-opioid receptor ligands on serum prolactin levels in rhesus monkeys

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Abstract

These studies investigated whether serum prolactin levels could be a quantitative marker of the apparent efficacy of κ-opioid receptor ligands in primates. The effects of s.c. bremazocine and U50,488 (*trans*-(+/−)-3,4-Dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide; agonists), nalorphine (partial agonist) and nalmefene (antagonist) on prolactin levels were studied in intact female rhesus monkeys. The above compounds, except nalmefene, increased prolactin levels, and their actions conformed to sigmoidal dose–effect curves. The rank order of the compounds' maximum effects in this neuroendocrine endpoint is similar to that in cloned κ-receptors in vitro, and in a presently studied thermal antinociception assay in vivo. Prolactin may therefore be a quantitative marker of the apparent efficacy of κ-opioid receptor ligands in primates.

Keywords: Prolactin; Nalmefene; Bremazocine; U50,488; Rhesus monkey; Efficacy

1. Introduction

Kappa (κ)-opioid receptor ligands may have pharmacotherapeutic potential in several conditions, including pain or hyperalgesia (e.g., Stein et al., 1989; Caudle et al., 1998) and substance abuse (e.g., Di Chiara and Imperato, 1988; Shippenberg et al., 1996; Mello and Negus, 1998; Kuzmin et al., 1998; Kreek et al., 1999; Nestby et al., 1999). However, synthetic high efficacy κ-opioid receptor agonists cause centrally mediated dysphoric and psychotomimetic effects in humans, and these effects limit clinical use of these compounds (Pfeiffer et al., 1981; Pande et al., 1996). The efficacy of κ-opioid receptor ligands at cloned human κ-opioid receptors has recently been studied in vitro, through measurement of the binding of [^{35}S]GTPgamma(γ)S, which is an indicator of agonist-induced activation of G-proteins (Zhu et al., 1997; Remmers et al., 1999). Information from these in vitro studies is of value in predicting in vivo κ-agonist efficacy, and thus the pharmacological profile of κ-opioid receptor ligands in human or non-human primates. For example, the synthetic κ-opioid receptor ligands bremazocine and U50,488 were high efficacy agonists, whereas nalorphine was a partial agonist in the [^{35}S]GTPγS assay (Zhu et al., 1997; Remmers et al., 1999). The clinically available opioid antagonist, nalmefene, only exhibited very low efficacy in the above in vitro studies (Remmers et al., 1999).

To date, few studies have compared quantitatively the apparent efficacy (or effectiveness) of κ-opioid receptor ligands in human or non-human primates, in part due to a lack of suitable in vivo assays. For example, some behavioral assays used to study in vivo κ-opioid receptor pharmacology in primates (e.g., antinociception and drug discrimination assays) have experimental cutoffs which limit the measurement of the apparent efficacy of these compounds. Kappa-opioid receptor agonists also cause neuroendocrine effects in human and non-human primates, including release of the anterior pituitary peptide hormone, prolactin (Gilbeau et al., 1986; Pfeiffer et al., 1986; Ur et al., 1997). Prolactin release from the anterior pituitary is under tonic inhibition by hypothalamic dopaminergic systems; it has been suggested that κ-opioid receptor agonists cause an increase in prolactin levels by modulating these dopaminergic systems (see Moore and Lookingland, 1995). The aim of the present studies was to determine whether prolactin levels could be used as an in vivo marker of the apparent efficacy of κ-opioid receptor ligands in primates

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(rhesus monkeys). The apparent efficacy profile of the κ-opioid receptor ligands in this neuroendocrine endpoint was compared to their profile in a test of thermal antinociception, which may be predictive of the analgesic actions of opioid compounds.

2. Materials and methods

2.1. Subjects

Intact, captive-bred rhesus monkeys (*Macaca mulatta*, 5 female, 2 male, all at least 5 years old) were used. The monkeys (weight range 5–8 kg) were singly housed with free access to water, and were fed appropriate amounts of monkey chow daily, supplemented by fruit two times per week. The subjects were maintained in accordance with the Institutional Animal Care and Use Committee of Rockefeller University, and guidelines of the Institute for Laboratory Animal Resources.

2.2. Procedures

2.2.1. Serum prolactin levels

Chair-trained female monkeys ($n = 4$) were tested during their follicular phase (days 2–12 of each 28-day cycle, as determined by the onset of visible bleeding). A single indwelling catheter (24 gauge; Angiocath, Becton Dickinson, Sandy, UT) was placed in a superficial leg vein, and secured with elastic tape. A multi-sample injection port (Terumo, Elkton, MD) was attached to the hub of the catheter; the port and catheter were flushed (0.3 ml of 50 U/ml heparinized saline) before use, and after each blood sampling. Approximately 15 min following catheter placement, two baseline blood samples were collected, 5 min apart from each other (defined as -10 and -5 min relative to the onset of dosing). At each sampling point, a 1.5-ml blood aliquot was placed in an EDTA vacutainer (not analyzed in the present studies). This was followed by a second 1.5-ml blood sample, which was placed in a plain vacutainer, and kept at room temperature until the time of spinning (3000 rpm at 4°C, for 5 min) and serum separation. Serum samples (approximately 400 μl) were kept at −40°C until the time of analysis. Samples were analyzed in duplicate with human prolactin radioimmunoassay kits (Nichols Diagnostics Institute, San Juan Capistrano, CA), following manufacturer's instructions. Standard calibration curves were determined for each kit with human prolactin standards (3–150 ng/ml). The intra-assay coefficient of variation for samples tested with the present kits was 5.3%, whereas the inter-assay coefficient of variation was 13.7%.

Monkeys were tested in a cumulative dosing design; cumulative s.c. dose–effect curves were carried out with a 30-min inter-injection interval, with doses increasing by 0.5 log units in each cycle. Samples were taken 20–25 min after each injection. In an antagonism experiment, a single dose of nalmefene (0.1 mg/kg, s.c.) was administered 30 min before re-determination of the U50,488 dose–effect curve.

Dose–effect curve data were analyzed with a four-parameter logistic equation, to yield a sigmoidal dose–effect curve (variable slope), with a non-linear regression program (Graphpad Prism, San Diego, CA). For the non-linear regressions, the lower end of the dose–effect curves was kept fixed at the mean baseline (pre-injection level), or at 3 ng/ml, if the baseline level was below the lowest prolactin standard. In potency and antagonism comparisons, individual log ED_{50} values and their 95% confidence limits (95% CL) were calculated by linear regression, from points above and below the 50% level of effect. The same pool of subjects was typically used for the pharmacological comparisons in this assay. The 0.05 α level was adopted in these studies; parameters (i.e., ED_{50} and maximum effect) were considered to be significantly different if their 95% CL did not overlap.

2.2.2. Thermal antinociception assay

The warm water tail withdrawal assay has been previously described (Dykstra and Woods, 1986). Briefly, the latency of chair-trained monkeys ($n = 4$–5; male and female) to remove their tail from a thermos flask containing warm water (either 40°C [non-noxious control temperature], 46° or 50°C [noxious temperatures]), was measured manually in 0.1 s increments up to a 20 s maximum cutoff. Consecutive tests in the same animal were separated by 2 min approximately, and the order of temperatures was varied among subjects and cycles. Following baseline latency determination, monkeys were tested in a cumulative s.c. dosing procedure identical to that described in the above neuroendocrine studies (i.e., testing at the three temperatures occurred between 20–25 min after each injection). Individual latency data were transformed into %maximum possible effect (%MPE) by the standard equation: %MPE = [(test latency-baseline latency)/(cutoff-baseline latency) × 100%]. Individual log ED_{50} values and their 95% CL were calculated by linear regression, as in the neuroendocrine assay, above.

2.3. Experimental design

Dose–effect curves for bremazocine (0.001–0.032 mg/kg [2.8–91 nmol/kg], U50,488 (0.032–1.0 mg/kg [0.069–2.1 μmol/kg] , nalorphine (0.1–3.2 mg/kg [0.2–9.1 μmol/kg]), nalmefene (0.032–1.0 mg/kg [0.085–2.6 μmol/kg]) and vehicle ($n = 4$–5) were studied in both neuroendocrine and antinociception assays. In an antagonism study, nalmefene (0.1 mg/kg) was administered before re-determination of the U50,488 dose–effect curve in the neuroendocrine assay. Consecutive experiments in the same subjects were separated by at least 72 h.

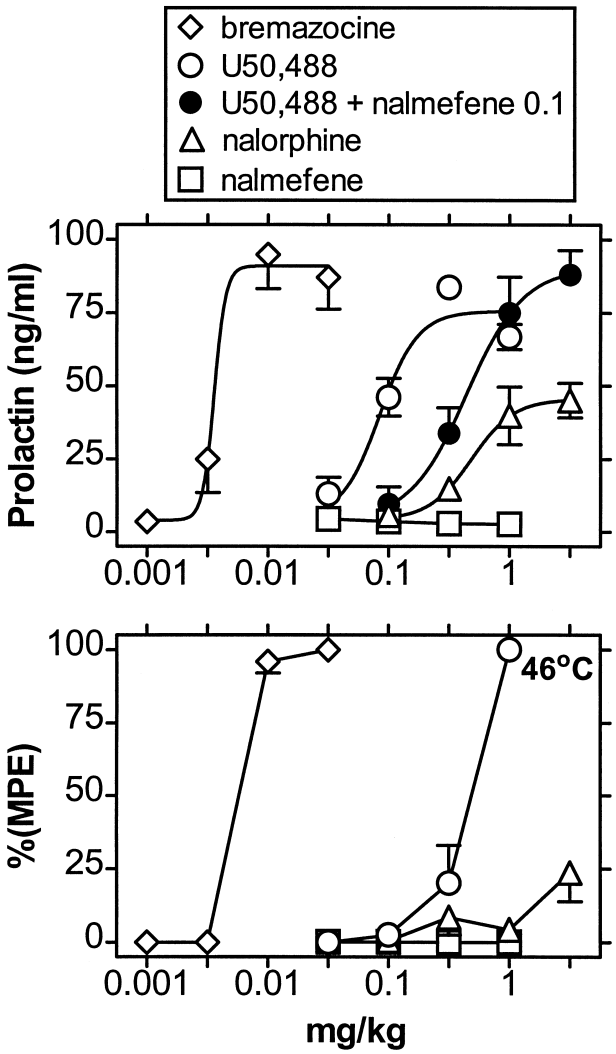

Fig. 1. Effects of κ-opioids on serum prolactin levels (upper panel) and antinociception against a 46°C thermal stimulus (lower panel) in rhesus monkeys ($n = 4–5$; mean ± S.E.M.). Abscissae: dose (mg/kg). Ordinates: (upper panel) serum prolactin levels (ng/ml); (lower panel) % maximum possible effect.

2.4. Chemicals

Bremazocine HCl (RBI, Natick, MA), U50,488 (*trans*-(+/−)-3,4-Dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methanesulfonate; RBI, Natick, MA), nalorphine HCl (RBI, Natick, MA) and nalmefene HCl (Baker Norton, Miami, FL) were dissolved in sterile water. Compounds were injected s.c. in the midscapular region of the back, typically in volumes of 0.1 ml/kg. All doses are expressed as the above forms of the compounds.

3. Results

3.1. Serum prolactin levels

Monkeys exhibited low pre-injection prolactin levels (typically 3 ng/ml or less); vehicle injection in five consecutive 30-min cycles did not elevate mean prolactin levels above 5 ng/ml ($n = 4$). Dose–effect curves are presented for bremazocine, U50,488, nalorphine and nalmefene ($n = 4$; Fig. 1). Nalmefene did not cause an increase in prolactin levels, up to the largest dose presently studied (1 mg/kg). By contrast, bremazocine, U50,488 and nalorphine caused dose-dependent increases in prolactin levels, and their effects were fitted to sigmoidal dose–effect curves by non-linear regression (see Table 1). Bremazocine and U50,488 both produced a significantly higher maximum plateau than nalorphine, but were not significantly different from each other. The potency (ED_{50}) order for the three above compounds on this neuroendocrine endpoint was bremazocine > U50,488 > nalorphine (Table 1).

Nalmefene (0.1 mg/kg) was administered 30 min before re-determination of the U50,488 dose–effect curve. This nalmefene pretreatment caused a significant and surmountable rightward shift in the U50,488 dose–effect curve (Fig. 1). The U50,488 ED_{50} value and [95% CL] in the presence of nalmefene (0.1 mg/kg) were 0.44 [0.23–0.84] mg/kg.

3.2. Thermal antinociception assay

Monkeys displayed a consistent pattern of pre-injection tail withdrawal latencies. They typically left their tails in the non-noxious water temperature (40°C) until cutoff, and removed their tails from noxious temperatures (46° and 50°C) within 2 s. Cumulative vehicle administration did

Table 1
Potency and effectiveness in neuroendocrine (prolactin release) and thermal antinociception assays ($n = 4–5$)

	Prolactin		Antinociception
	Maximum effect (ng/ml [95%CL])	ED_{50} (mg/kg [95%CL])	ED_{50} (mg/kg [95%CL])
Bremazocine	90.1 [70.4–111.4]	0.0044 [0.0023–0.0084]	0.0058 [0.0053–0.0063]
U50,488	75.6 [64.8–86.4]	0.087 [0.064–0.12]	0.43 [0.35–0.77]
Nalorphine	45.0 [31.5–60.1]	0.58 [0.21–1.57]	n/a[a]
Nalmefene	Ineffective	–	Ineffective

[a] Nalorphine ED_{50} in antinociception assay could not be calculated, as 50% effect was not reached up to the largest dose studied (3.2 mg/kg).

not cause an elevation in tail withdrawal latencies at the two noxious temperatures ($n = 5$). Dose–effect curves for bremazocine, U50,488, nalorphine and nalmefene were compared in the same group of subjects ($n = 4–5$; Fig. 1). Nalmefene, up to the largest dose presently studied, was ineffective against 46° or 50°C thermal stimuli. Nalorphine was modestly effective against the 46°C stimulus (Fig. 1), and was ineffective against the 50°C stimulus (not shown), up to the largest dose presently studied (3.2 mg/kg). This nalorphine dose (3.2 mg/kg) produced a significant increase in % MPE against the 46°C stimulus, compared to vehicle injection ($n = 5$; Wilcoxon signed ranks test = 15; $p < 0.04$). By contrast, both bremazocine and U50,488 were fully effective against the 46°C stimulus (see Fig. 1 and Table 1), as well as against the 50°C stimulus (data not shown).

4. Discussion

These studies illustrate that prolactin levels can be used as a sensitive, quantitative neuroendocrine marker for the apparent efficacy of κ-opioid receptor ligands in primates. It should be noted however, that ligands acting on other receptors (including μ-opioid and serotonergic receptors) also cause increases in prolactin levels (see Moore and Lookingland, 1995, for review). Sigmoidal dose–effect curves were observed for bremazocine, U50,488 and nalorphine in this neuroendocrine endpoint, and this allowed direct observation of their apparent efficacy in vivo. The presently obtained rank order of apparent efficacy on the neuroendocrine endpoint (i.e., bremazocine = U50,488 > nalorphine > nalmefene), is similar to the rank order for the efficacy of these compounds at cloned human κ-receptors in vitro (Remmers et al., 1999).

The rank order of potency and effectiveness of the ligands in the neuroendocrine assay are also consistent with those observed in the present antinociception assay. Furthermore, a similar potency order was previously observed in a κ-opioid receptor agonist drug discrimination assay in rhesus monkeys (France et al., 1994). The lack of effectiveness of nalmefene in the neuroendocrine endpoint was not due to limitations on dosing, since nalmefene (0.1 mg/kg) caused a significant shift in the U50,488 dose–effect curve. This indicates that nalmefene can produce antagonist (but not agonist) effects at the κ-opioid receptor population that mediates U50,488-induced prolactin release. Nalmefene also attenuated the prolactin-releasing effects of the opioid peptide dynorphin A-(1–13) in humans (Kreek et al., 1999). The nalmefene pretreatment dose used above (0.1 mg/kg) was also sufficient to cause antagonism of a κ-opioid receptor mediated effect in the thermal antinociception assay in rhesus monkeys (France and Gerak, 1994).

Up to the largest dose presently studied, and in a previous report (France et al., 1994), nalorphine was only modestly effective or ineffective in the assay of thermal antinociception. By contrast, the complete nalorphine dose–effect curve could be described in the present neuroendocrine endpoint. This suggests that in rhesus monkeys, prolactin levels are a more sensitive endpoint for κ-opioid receptor agonist effects than thermal antinociception.

Overall, the present studies indicate that serum prolactin levels may be a sensitive neuroendocrine marker for the potency and apparent efficacy of systemically administered κ-opioid receptor ligands in primates. Due to the non-invasive and quantitative nature of this endpoint, prolactin levels may be a suitable marker for the in vivo pharmacology of novel synthetic or peptidic κ-opioid receptor ligands (e.g., Butelman et al., 1999; Kreek et al., 1999) in human or non-human primates. Such a marker could be useful in the development of these ligands for the pharmacotherapeutic management of pain and substance abuse.

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References

Butelman, E.R., Harris, T., Perez, A., Kreek, M.J., 1999. Effects of systemically administered dynorphin A(1–17) in rhesus monkeys. J. Pharmacol. Exp. Ther. 290, 678–686.

Caudle, R.M., Finegold, A.A., Mannes, A.J., Tobias, M.D., Kenshalo, D.R., Iadarola, M.J., 1998. Spinal k_1 and k_2 opioid binding sites in rats, guinea pigs, monkeys and humans. NeuroReport 9, 2523–2525.

Di Chiara, G., Imperato, A., 1988. Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. J. Pharmacol. Exp. Ther. 244, 1067–1080.

Dykstra, L.A., Woods, J.H., 1986. A tail withdrawal procedure for assessing analgesic activity in rhesus monkeys. J. Pharmacol. Methods 15, 263–269.

France, C.P., Gerak, L.R., 1994. Behavioral effects of 6-methylene naltrexone (nalmefene) in rhesus monkeys. J. Pharmacol. Exp. Ther. 270, 992–999.

France, C.P., Medzihradsky, F., Woods, J.H., 1994. Comparison of kappa opioids in rhesus monkeys: behavioral effects and receptor affinities. J. Pharmacol. Exp. Ther. 268, 47–58.

Gilbeau, P.M., Hosobuchi, Y., Lee, N.M., 1986. Dynorphin effects on plasma concentrations of anterior pituitary hormones in the nonhuman primate. J. Pharmacol. Exp. Ther. 238, 974–977.

Kreek, M.J., Schluger, J., Borg, L., Gunduz, M., Ho, A., 1999. Dynorphin A(1–13) causes elevation of serum levels of prolactin through an opioid receptor mechanism in humans: gender differences and implications for modulation of dopaminergic tone in the treatment of addictions. J. Pharmacol. Exp. Ther. 288, 260–269.

Kuzmin, A.V., Gerrits, M.A.F.M., Van Ree, J.M., 1998. κ-opioid receptor blockade with nor-binaltorphimine modulates cocaine self-administration in drug-naïve rats. Eur. J. Pharmacol. 358, 197–202.

Mello, N.K., Negus, S.S., 1998. Effects of kappa opioid agonists on

cocaine- and food-maintained responding in rhesus monkeys. J. Pharmacol. Exp. Ther. 286, 812–824.

Moore, K.E., Lookingland, K.J., 1995. Dopaminergic neuronal systems in the hypothalamus. In: Bloom, F.E., Kupfer, D.J. (Eds.), Psychopharmacology: the fourth generation of progress. Raven Press, New York, pp. 245–256.

Nestby, P., Schoffelmeer, A.N.M., Homberg, J.R., Wardeh, G., De Vries, T.J., Mulder, A.H., Vanderschuren, L.J.M.J., 1999. Bremazocine reduces unrestricted free-choice ethanol self-administration in rats without affecting sucrose preference. Psychopharmacology 142, 309–311.

Pande, A.C., Pyke, R.E., Greiner, M., Wideman, G.L., Benjamin, R., Pierce, M.W., 1996. Analgesic efficacy of enadoline versus placebo or morphine in postsurgical pain. Clin. Neuropharmacol. 19, 451–456.

Pfeiffer, A., Pasi, A., Mehraein, P., Herz, A., 1981. Psychotomimesis mediated by k opiate receptors. Science 233, 774–776.

Pfeiffer, A., Braun, S., Mann, K., Meyer, H.D., Brantl, V., 1986. Anterior pituitary responses to a kappa opioid agonist in man. J. Clin. Endocrinol. Metab. 62, 181–185.

Remmers, A.E., Clark, M.J., Mansour, A., Akil, H., Woods, J.H., Medzihradsky, F., 1999. Opioid efficacy in a C6 glioma cell line stably expressing the human kappa opioid receptor. J. Pharmacol. Exp. Ther. 288, 827–833.

Shippenberg, T.S., Lefevour, A., Heidbreder, C., 1996. Kappa-opioid receptor agonists prevent sensitization to the conditioned rewarding effects of cocaine. J. Pharmacol. Exp. Ther. 276, 545–554.

Stein, C., Millan, M.J., Shippenberg, T.S., Peter, K., Herz, A., 1989. Peripheral opioid receptors mediating antinociception in inflammation. Evidence for involvement of mu, delta and kappa receptors. J. Pharmacol. Exp. Ther. 248, 1269–1275.

Ur, E., Wright, D.M., Bouloux, M.G., Grossman, A., 1997. The effects of spiradoline (U62,066E), a k-opioid receptor agonist, on neuroendocrine function in man. Br. J. Pharmacol. 120, 781–784.

Zhu, J., Li, J.G., Chen, C., Liu-Chen, L.Y., 1997. Activation of the cloned human kappa opioid receptor by agonists enhances [^{35}S] GTP gamma S binding to membranes: determination of potencies and efficacies of ligands. J. Pharmacol. Exp. Ther. 282, 676–684.