IN VIVO DETERMINATION OF μ OPIOID RECEPTOR RESERVE AND TURNOVER IN RHESUS MONKEYS AFTER IRREVERSIBLE BLOCKADE WITH CLOCINNAMOX

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The two prototypical μ opioid agonists morphine and alfentanil were tested in a warm-water tail withdrawal antinociception assay performed at 45°C, 50°C and 55°C in rhesus monkeys before and after administration of the irreversible opioid antagonist clocinnamox (0.1 mg/kg s.c.). Clocinnamox acutely decreased the available opioid receptors by up to 90%. Receptor numbers returned to control levels with a half-life of approximately one week. The affinity, efficacy, the maximum effect, and the stimulus-response transduction factor did not change significantly over time. The efficacy of alfentanil was always 2-3-fold higher than that for morphine, the efficacies of both agonists being higher at lower temperatures. The affinity of alfentanil was 29-fold higher than that of morphine.

The ability of clocinnamox, a 14-cinnamoyl-amino-morphinone (1), to inhibit the effects of different μ opioid agonists has been demonstrated both in mice (2) and rhesus monkeys (3) in a variety of behavioral procedures. In the mouse warm-water tail withdrawal test (2), clocinnamox produced a rightward shift and a depression of the dose-response curves for both morphine and fentanyl, strongly indicating that clocinnamox acted as an irreversible blocker at μ opioid receptors. Partial irreversible inactivation of opioid receptors by clocinnamox was used in the present study to calculate the fraction of available receptors, q; the efficacy, e; the in vivo dissociation constant, K_A; and the stimulus-response transduction factor; for the two selective μ agonists morphine and fentanyl according to the method of Furchgott (4) as modified by Black & Leff (5). To that end, dose-response curves were obtained for alfentanil and morphine in a warm-water tail withdrawal antinociception assay at 45°C, 50°C, and 55°C in rhesus monkeys before and after administration of 0.1 mg/kg clocinnamox (s.c.) which produced an acute rightward shift of the dose-response curves of both aggonists at all temperatures. In addition, the maxima of the dose-response curves of both agonists were depressed at all temperatures tested.

The fraction of receptors available for alfentanil at 50°C showed the following time course (control q value =1): 3 h after clocinnamox administration, 0.11; 1 d, 0.11; 3 d, 0.041; 7 d, 0.22; 14 d, 0.79; and 21 d, 0.58; at 55°C: 3 h, 0.12; 1 d, 0.081; 3 d, 0.091; 7 d, 0.74; 14 d, 1.48; and 21 d, 0.94. Assuming monoexponential reappearance kinetics, the overall opioid receptor recovery half-life was 6.3 days (r^2=0.86); the respective half-life for 55°C alone was 7.7 days (r^2=0.64). When morphine was tested using a 2-day interval, the respective q values after 0.1 mg/kg clocinnamox could not be calculated up to day 6 because the morphine dose-response curves were so severely depressed. From day 6 on, the q values for morphine were, at 50°C: 6 d, 0.18; 8 d, 0.29; 10 d, 0.19; 12 d, 0.17; 14 d, 0.14; 21 d, 0.51; 28 d, 0.39; and 49 d, 0.56. The respective q values at 55°C were: 6 d, 0.062; 8 d, 0.064; 10 d, 0.085; 12 d, 0.059; 14 d, 0.12; 21 d, 0.076; 28 d, 0.44; 49 d, 0.12. When morphine was tested using a 7-day interval, the respective q values after 0.1 mg/kg clocinnamox were, at 45°C: 4 h, 0.41; 7 d, 0.94; 21 d, 3.8; 28 d, 1.7; and 35 d, 2.8. The respective q values for morphine at 50°C were: 4 h, 0.88; 7 d, 0.61; 14 d, 3.2; 21 d, 4.3; 28 d, 4.2; 35 d, 1.7; and 42 d, 0.89. As morphine had caused considerable respiratory depression at very high doses when tested using the 2-day interval, dose-response curves for morphine at 55°C using the 7-day interval were not followed up to doses at which morphine's antinociceptive effect leveled off. Therefore, no q values can be given for this set of experiments.To summarize, when the 2-day test interval was used for assessing morphine's effects before and after administration of the irreversible opioid antagonist clocinnamox, the receptor population did not recover to control levels within the test period. If, however, morphine was tested using a 7-day interval, the receptor population not only recovered to control levels but also showed an overshoot, similar to that found for alfentanil.These data indicate that the assessment of an irreversible μ opioid antagonist's effect by frequent testing with a low-efficacy μ opioid agonist might result in a distortion by agonist-tolerance-induced receptor population changes. When care was taken to minimize this distortion, 0.1 mg/kg clocinnamox was shown to initially decrease the receptor population by up to 90%; the receptor population recovered with an approximate half-life of one week, showed an overshoot 2-4 weeks after clocinnamox administration and returned to pre-clocinnamox levels afterwards.

In contrast to the distinct time dependence of q, alfentanil affinity (as expressed by the in vivo dissociation constant of the agonist, K_A); its efficacy, e; the theoretically obtainable maximum effect of the μ opioid antinoceptive system, E_m; and the stimulus-response transduction factor did not change significantly over time. The respective values are shown in the Table. The same pattern was obtained with morphine.Overall, alfentanil showed a 29-fold higher affinity than morphine regardless of the temperature tested, the respective K_A values being 0.84 mg/kg for alfentanil and 24 mg/kg for morphine. The affinities of alfentanil and morphine did not change with temperature. The efficacy of alfentanil was always 2-3-fold higher than that of morphine for any temperature tested. Ihe efficacies of both μ opioid agonists were higher at lower temperatures, confirming earlier qualitative work (6). These findings suggest that a 3-4-fold larger fraction of μ opioid receptors has to be occupied to counteract the thermonociceptive stimulus at 55°C than at 50°C. E_m and the stimulus-response transduction factor did not differ between the two agonists. Morphine had been used in similar studies by a different group in rats using the same behavioral paradigm using buprenorphine as the (pseudo)irreversible antagonist (7, 8); the values for K_A and e were almost identical. A detailed report has been submitted for publication (9).

Table. Efficacy, in vivo affinity (expressed as the in vivo dissociation constant, K_A), theoretically attainable maximum effect (E_m), baseline withdrawal latency, and stimulus-response transduction factor for alfentanil and morphine in the rhesus monkey warm-water tail withdrawal assay. Values are means ± S.E.M. of n determinations. The p values were obtained by one-way analysis of variance (ANOVA) and quantitatively state the probability that the the variation among the column means is caused by chance.

Drug	**Alfentanil**		**Morphine**			**P value (ANOVA)**
Temperature	**50°C** (n=8)	**55°C** (n=6)	**45°C** (n=7)	**50°C** (n=17)	**55°C** (n=9)	
Efficacy	32 ± 1	8 ± 1	15 ± 3	10 ± 1	3 ± 1	<0.0001
K_A [log,mg/kg]	-0.19 ± 0.12	0.08 ± 0.15	1.12 ± 0.17	1.45 ± 0.09	1.41 ± 0.22	<0.0001
K_A [mg/kg]	0.65	1.2	13	28	26	
E_m [s]	23 ± 1	25 ± 2	20 ± 1	23± 1	21 ±1	0.11
baseline latency [s]	1.9 ± 0.6	0.9 ± 0.1	1.6 ± 0.1	1.6 ± 0.2	1.0 ± 0.1	0.13
transduction factor	1.6 ± 0.3	1.8 ± 0.2	2.0 ± 0.3	2.0 ± 0.2	1.4 ± 0.2	0.33

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