Pharmacology Biochemistry and Behavior, Vol. 58, No. 1, pp. 189–194, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(96)00480-7

Influence of Continuous Levels of Fentanyl in Rats on the μ-Opioid Receptor in the Central Nervous System

ERIKA ALBRECHT,* NADJA HEINRICH,* DOROTHEA LORENZ,* INGO BAEGER,* NELYA SAMOVILOVA,† KLAUS FECHNER* AND HARTMUT BERGER*

** Department of Peptide Pharmacology, Research Institute of Molecular Pharmacology, D-10315 Berlin, F.R.G.*
† Cardiology Research Centre, Academy of Medical Sciences, Moscow, Russia

Received 7 August 1996; Revised 18 October 1996; Accepted 18 October 1996

ALBRECHT, E., N. HEINRICH, D. LORENZ, I. BAEGER, N. SAMOVILOVA, K. FECHNER AND H. BERGER. *Influence of continuous levels of fentanyl in rats on the μ-opioid receptor in the central nervous system.* PHARMACOL BIOCHEM BEHAV **58**(1) 189–194, 1997.—The highly potent and efficacious μ-opioid agonist fentanyl was SC infused into rats with submaximal analgesic doses (0–1.14 μmol/kg/day) continuously for 8 days, checked by the constant daily urinary recovery of intact drug (0.43 ± 0.031% of the daily dose). Tail-flick latencies measured at 24 (day 1) and 48 h (day 2) after starting the infusion were increased in a dose-dependent fashion compared with those before the infusion (day 0). However, at day 8, the latencies were increased only weakly, not significantly, revealing tolerance to the antinociceptive activity of fentanyl. Fentanyl at all doses showed no significant effect on the capacity (B_{max}) and affinity (K_d) of the μ-opioid receptor binding of DAMGO to whole brain (B_{max} 126.2 ± 3.00 fmol/mg protein, K_d 1.00 ± 0.04 nM) and spinal cord (B_{max} 48.24 ± 2.71 fmol/mg protein, K_d 1.93 ± 0.13 nM) membranes gained from the rats after killing them at day 8. Gpp(NH)p increased the K_d for brain and spinal cord sites by 3.09 and 2.65, respectively, independent of the fentanyl dose. The infusion with fentanyl did not alter the basal and forskolin-stimulated adenylate cyclase activity in the whole brain membranes, nor did it change the inhibition of the forskolin-stimulated activity by DAMGO. It is concluded that, in rats, constant long-term body levels of highly potent μ-agonists result in a tolerant state that, however, does not produce overall changes in the parameters of their specific receptor sites in the CNS, i.e., receptor capacity and affinity, and in the events closely related to them, i.e., their regulation by GTP and of adenylate cyclase. This does not exclude such possible changes to be restricted to specific regions in the CNS. © 1997 Elsevier Science Inc.

Rat μ-Opioid receptor Fentanyl DAMGO binding Analgesia GTP regulation Tolerance Adenylate cyclase

THE biochemical mechanisms by which opiate agonists induce tolerance remain poorly understood. Because binding of the opioid drugs to their receptors in the central nervous system (CNS) is the first step in the chain of molecular events leading to the observed analgesic effect, changes in the concentration and/or affinity of the receptors have been expected to explain the decline in the effect, i.e., tolerance, at long-term stimulation of the receptors by the drugs.

However, studies on the opioid receptor in the CNS of laboratory animals after long-term opioid administration, rendering the animals tolerant to the analgesic effect of opioids, gave conflicting results. Using rats or guinea pigs and μ-opioid receptor agonists as tolerance-inducing drugs in vivo and ligands for receptor binding in vitro, elevation (1,5,19,20,26), decrease (2,15,26,28), or no change (8,15,27) in the capacity of the μ-receptors in brain and spinal cord preparations were found, the results for a certain treated animal group sometimes even depending on the conditions in the binding experiments (17). In these studies, very seldom did the affinity of the μ-receptor in tolerant rats differ from that in control rats (19).

Furthermore, instead of changes in the receptor capacity or affinity, changes in the regulation of the receptor binding

Requests for reprints should be addressed to Hartmut Berger, Research Institute of Molecular Pharmacology, Alfred-Kowalke-Str. 4, D-10315 Berlin, F.R.G.

by GTP (27,28) and in the activity of the adenylate cyclase (10) or inhibition of the enzyme by the opioids (10,26) have been sought as being involved in the development of tolerance in laboratory animals.

Nearly all of these studies used intermittent administration of high doses of morphine to introduce a high degree of tolerance to its antinociceptive effect for the subsequent biochemical investigations. Differences in experimental protocol, and different and fluctuating body levels of morphine should result in varying degrees of tolerance, and might be partly responsible for the differing results.

The objective of this study was, therefore, to investigate the influence of different doses of a μ-opioid agonist under controlled conditions on the μ-opioid receptor in the rat CNS. For this purpose, the highly potent and efficacious μ-agonist fentanyl was infused continuously into rats at submaximal analgesic doses for subsequent study of the effects on the μ-receptor binding, its regulation by GTP, and the activity of the adenylate cyclase in membrane fractions obtained from brain and spinal cord.

METHOD

Materials

Fentanyl citrate, levorphanol, 5′-guanylylimidodiphosphate (Gpp(NH)p, theophylline, ethylene glycol bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), creatine phosphate, adenosine-5′-triphosphate (ATP), guanosine-5′-triphosphate (GTP), forskolin, and creatine phosphokinase (CPK) from rabbit muscle were purchased from Sigma Chemie (F.R.G.). [D-Ala(2), N-Me-Phe(4), Gly(5)-ol)]-enkephalin (DAMGO) and dithiothreitol (DTT) were from Serva (F.R.G.). [^{3}H]-DAMGO (2.22 TBq/mmol) and the cyclic adenosine-3′:5′-monophosphate (cAMP) kit were from Amersham, Inc. (England).

Infusion of Rats with Fentanyl

In eight experiments, a total number of 59 male Wistar rats (Møellegaard Breeding Centre, Denmark) was anaesthetized by pentobarbital sodium (50 mg/kg IP) and implanted with ALZET osmotic minipumps (ALZA Corporation, USA) model 2001 into the nuchal region. The pumps were filled with physiological saline (control rats, $n = 15$) or solutions of different concentrations of fentanyl to give continuous delivery of the drug up to 1.14 μmol (600 μg) of fentanyl citrate per kg body weight and 24 h (0.14 μmol, $n = 15$; 0.21 μmol, $n = 10$; 0.28, 0.57, 0.85, and 1.14 μmol, $n = 5$ each). The constant rate of infusion for 7 days was checked by determination of the urinary excretion of fentanyl (see below).

Assessment of Urinary Fentanyl

Rat urine samples were adjusted to pH 6.0 with concentrated phosphoric acid and applied to XtrackT® cartridges (AMCHRO) conditioned with each 6 ml of methanol and 0.05 M potassium phosphate (pH 6.0). After washing the cartridges with, consecutively, water (6 ml), 0.05% trifluoroacetic acid in water (6 ml), methanol (18 ml), and 0.5% ammonia in isopropanol (4 ml), fentanyl was eluted with 1.0% ammonia in acetonitrile (4 ml). The eluates were dried by vacuum evaporation. The residues were dissolved in 32.5% acetonitrile/67.5% 0.01 M triethylammonium phosphate, pH 7.0 (medium A), and applied to a LiChrospher100 RP-18 5-μm endcapped column (125 × 4 mm) with precolumn (10 × 4 mm). Low-pressure discontinuous gradient elutions, using WATERS pump 610, WATERS valve station 610 and 600E controller, were run with medium A and 70% acetonitrile/30% 0.01 M triethylammonium phosphate, pH 7.0, starting with 100% medium A (4 min), and followed by 80, 10, and 100% medium A for 16, 15, and 20 min, respectively, at a flow rate of 1 ml/min. Fentanyl was detected at a retention time of 22.7 min with a WATERS 486 detector at 205 nm. Recovery of fentanyl from urinary samples spiked with the drug was 90.6 ± 7.3% (mean ± SD).

For the determination of the amount of fentanyl that was excreted into urine, 10 rats were injected SC with bolus doses of fentanyl citrate of 8–70.5 μg/kg body weight, and 24 h samples of urine were collected for 3 days. The low doses used should comply with the blood levels of fentanyl reached with the rats infused continuously with fentanyl.

Test for Analgesia

Immediately before (day 0) and on days 1, 2, and 8 after implantation of the minipumps, the animals were tested for analgesia by the tail flick method using a beam (analgesiometer from Technical & Scientific Equipment GmbH, Germany). From the test latency (TL), baseline latency before implantation of the pumps (BL, being about 3.5 s), and a cut-off time of the beam of 10 s, the measured analgesia was expressed as percentage of the maximum possible effect (MPE) according to $\%MPE = 100(TL - BL)/(10 - BL)$. The analgesic parameters were compared over the dose groups using one-way ANOVA followed by post hoc tests (Student–Newman–Keuls test) (29).

Membrane Preparation

At the end of the infusion period at day 8, before the decline in the infusion rate, the rats were killed by decapitation and the whole brain without cerebellum and the spinal cord were removed, frozen in liquid nitrogen, and stored at −70°C. All following steps were performed at 0–4°C. For receptor binding, the brain tissue obtained from each individual rat was homogenized by 10 strokes with a teflon-glass homogenizer (Potter S, Braun, Melsungen, F.R.G.) in 50 mM Tris/HCl buffer, pH 7.4, at 0.1 g wet weight/ml. After centrifugation at 1,000 × *g* for 5 min, the supernatant above the pellet P1 was centrifuged at 26,000 × *g* for 20 min, resulting in pellet P2.

The P2 pellet from each rat was washed three times by rehomogenization by hand with 24 ml of buffer and centrifugation at 26,000 × *g* for 20 min to release the endogenously bound fentanyl. Given the pharmacokinetic parameters for fentanyl infused continuously into rats [steady-state blood level, steady-state brain/blood partition coefficient 4.0, from (3)], the maximal possible concentration of fentanyl in the brain at the highest infusion dose (1.14 μmol/kg/day) was estimated to be about 50 pmol per g tissue. When the brain membrane fraction from untreated rats was incubated at 25°C with fentanyl at a concentration 10-fold the above estimated value, the washing procedure used was shown to be necessary and sufficient for the receptor binding not to be influenced by any residual fentanyl.

Using spinal cord, it was found that a great part of μ-receptor binding sites was bound to the P1 pellet. Therefore, the spinal cord homogenate was exposed immediately to the high-speed centrifugation and the pellet representing the combined P1/P2 was used for the experiments.

Membrane protein content was determined according to (4), using bovine serum albumin as standard.

μ-Receptor Binding of DAMGO

About 500–800 μg of membrane protein of brain or spinal cord were incubated in triplicates with 130,000 dpm/ml (1 nM) of the μ-opioid receptor ligand [^{3}H]-DAMGO in absence and presence of 10 different concentrations (0.1 up to 100 nM) of unlabeled DAMGO in 50 mM Tris-HCl buffer (pH 7.4) at 25°C for 1 h. These conditions were shown to be sufficient to reach receptor-binding equilibrium with no loss of binding sites. Nonspecific binding (<10 and <30% of total binding for brain and spinal cord preparations, respectively) was determined from incubations including 20 μM levorphanol. In some studies, 70 μM Gpp(NH)p was included in the incubation to look for change in receptor affinity. For spinal cord membranes, values with and without Gpp(NH)p were compared in pooled samples.

At the end of incubation, the samples were diluted with buffer and immediately filtered and washed with 3 × 3 ml Tris-buffer through Whatman GF/B filters in a Brandel-Harvester. The filters with the pelleted membranes were counted for ^{3}H-activity. The receptor-binding parameters, i.e., the dissociation constant K_d and the binding capacity B_{max}, were estimated using the program LIGAND (BIOSOFT, Cambridge, UK).

Data for K_d and B_{max} obtained without Gpp(NH)p and with/without Gpp(NH)p were analyzed with, respectively, one-way multivariate ANOVA and ANOVA for a double multivariate repeated measure design, with the dependent variables K_d and B_{max} and the factor variables in vivo fentanyl dose and in vitro Gpp(NH)p treatment (29).

Adenylate Cyclase Assay

The membrane fractions from the control rats and the rats receiving 1.14 μmol of fentanyl/kg/day were, after thawing, rehomogenized by Ultra-Turrax (Janke & Kunkel, F.R.G.). One-hundred-microliter quadruplicates of the membranes (2.5 μg of protein per tube) were incubated in 50 mM Tris/HCl buffer (pH 7.4) in presence of 60 mM NaCl, 2.5 mM $MgCl_2$, 1 mM DTT, 0.5 mM EGTA, 0.5 mM ATP, ATP generating system (20 mM creatine phosphate and 10 U of CPK), 10 mM theophylline, and 20 μM GTP for 5 min at 25°C. Forskolin (10 μM) was included in part of the incubations for stimulating the activity and the basal as well as forskolin-stimulated activity was measured, additionally, in presence of 10 μM DAMGO. The reaction was stopped by heating the samples for 3 min at 95°C. cAMP was determined by the binding assay.

The data were analyzed with three-way repeated measures ANOVA (29) with one between-subjects-factor (control/fentanyl-treated rats) and two cross-classified within-subjects-factors (enzyme activity basal/forskolin-stimulated and without/with DAMGO).

Statistics

The statistics used are described separately for the different parts of the study (see above). Numerical data in the text and tables and bars in the figures are expressed as the mean ± SEM. All computations were performed with the program SPSS for Windows, Release 6.1 (SPSS Inc., Chicago, IL, USA) or STATISTICA for Windows, Release 4.5 (StatSoft, Inc., Tulsa, OK, USA).

RESULTS

Of a subcutaneously injected dose of fentanyl, only 0.95 ± 0.20% was recovered in rat urine within 24 h, without any drug being detected after this time. Nevertheless, this low amount was sufficient for urinary excretion to be measured in rats implanted with minipumps containing fentanyl. From the calculated dose delivered every day from day 1 after implantation onward and from the corresponding amount of fentanyl determined in the daily collected urine, the daily recovery of fentanyl was estimated to be 0.43 ± 0.031% of the daily dose and, more importantly, this was shown to be constant for all doses with time from day 1, the time the minipump reached its constant level of delivery (Fig. 1).

Evaluation of %MPE data by ANOVA showed high significant dependence of the antinociceptive effect of fentanyl on its dose at day 1 (Fig. 2) and day 2 (data not shown) after the beginning of the infusion ($F(6,52) = 10.27$, $p < 0.001$), and, by Student–Newman–Keuls post hoc test, the antinociceptive response became significant from 0.57 μmol/kg/day upward (Fig. 2). However, at day 8, the response was not significantly different between all doses, but was significantly lower when compared with the response at day 1 (Fig. 2) or day 2 for all doses that had lead to significant analgesia at these days, revealing tolerance (Fig. 2).

Using the program LIGAND, a one-site binding model

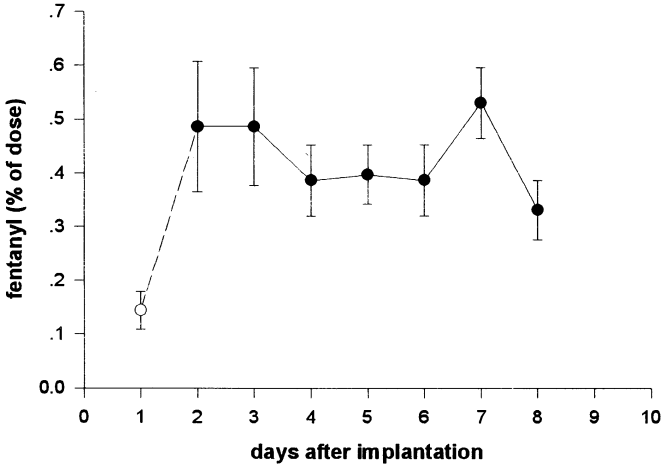

FIG. 1. Daily (24 h) recovery of fentanyl (% of daily dose ± SEM) in urine of rats ($n = 8$) implanted with osmotic minipumps delivering 0.14–0.57 μmol fentanyl/kg/day. The value given for day 1 (0–24 h) after implantation is an apparent one because it is related to the constant delivery rate of the pumps reached only after about 1 day after implantation.

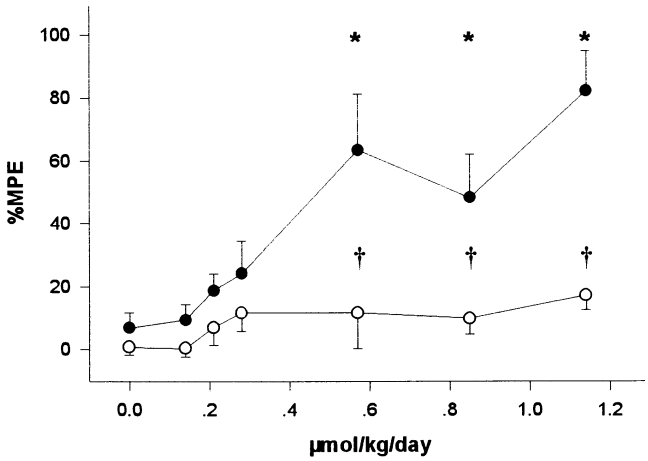

FIG. 2. Dependence of analgesia in rats (n = 59) on the dose of fentanyl daily delivered from osmotic minipumps continuously for 8 days. Tail-flick latencies were measured at day 1 (●) and day 8 (○) after starting the infusion (day 0) and are given as mean ± SEM of %MPE. *$p < 0.05$ vs. control (dose 0) and †$p < 0.05$ vs. day 1 by Student–Newman–Keuls test.

gave the best fit for the data of the binding of the μ-agonist DAMGO to the receptors in the brain and spinal cord preparations with and without Gpp(NH)p. Table 1 summarizes the binding constants and capacities of the membrane preparations from the rats infused with different doses of fentanyl.

ANOVA showed no significant effect of the in vivo fentanyl dose on K_d and B_{max} for the in vitro brain preparations as well as for those of spinal cord, and no interaction between fentanyl dose and Gpp(NH)p influence on K_d and B_{max}. However, the influence of Gpp(NH)p on the K_d values, by univariate analysis, was highly significant ($F(1,17) = 155.95$, $p < 0.001$ for brain; $F(1,5) = 11.62$, $p < 0.05$ for spinal cord; Table 1). For the whole group of control rats and rats infused with various doses of fentanyl, the mean values of K_d (nM) and B_{max} (fmol/mg protein) of the binding of DAMGO to the μ-receptors in the brain were 1.00 ± 0.04 and 126.2 ± 3.00, and in spinal cord 1.93 ± 0.13 and 48.24 ± 2.71, respectively. Gpp(NH)p increased the K_d for brain and spinal cord sites by 3.09 and 2.65, respectively.

Evaluating the data for the adenylate cyclase activity by ANOVA, a significant influence of DAMGO ($F(1,36) = 25.50$, $p < 0.001$) and forskolin ($F(1,36) = 802.06$, $p < 0.001$) on the activities in the brain membranes and a significant influence of forskolin on the inhibition by DAMGO ($F(1,36) = 22.26$, $p < 0.001$) was found (Table 2). However, the infusion of the rats with fentanyl did not produce significant changes in any of the activities (basal or in presence of DAMGO, forskolin, or DAMGO/forskolin) of the adenylate cyclase compared with those measured in the membranes of control rats ($F(1,36) = 1.306$, $p = 0.26$).

DISCUSSION

In this study, rats were administered a μ-opioid agonist over a period of 8 days under conditions that should minimize the development and expression of tolerance to its analgesic action. First of all, fentanyl, a μ-agonist of high analgesic activity, was used. Such a high-efficacy opioid is assumed to produce smaller degrees of tolerance than opioids with lower analgesic potency and efficacy like morphine, the most widely

TABLE 1

μ-OPIOID RECEPTOR BINDING OF DAMGO TO MEMBRANE FRACTIONS OF BRAIN (WITHOUT CEREBELLUM) AND SPINAL CORD OF MALE RATS AFTER THEIR CONTINUOUS S.C. INFUSION WITH 0 TO 1.14 μMOL FETANYL/KG BODY WEIGHT/DAY FOR 8 DAYS

		Brain		Spinal cord	
In vivo fentanyl (μmol/kg/day)	Gpp(NH)p 70 μM	Kd (nM)	Bmax (fmol/mg)	Kd (nM)	Bmax (fmol/mg)
0 (vehicle)	−	0.88 ± .036	122.3 ± 4.00	1.79 ± 0.183	46.31 ± 3.14
	+	3.29 ± .121***	117.5 ± 5.74	5.99 ± 1.267*	48.30 ± 6.55
0.14	−	1.00 ± .145	131.0 ± 9.30		
0.28	−	1.12 ± .177	130.4 ± 5.81		
0.57	−	1.05 ± .134	137.8 ± 13.36		
0.85	−	1.18 ± .182	112.8 ± 9.87	2.02 ± 0.383	54.66 ± 7.77
	+	2.79 ± .407***	99.9 ± 6.83	3.96 ± 0.411*	50.20 ± 3.04
1.14	−	1.00 ± .085	131.7 ± 3.25	2.13 ± 0.029	45.67 ± 5.57
	+	3.00 ± .217***	125.0 ± 4.84	4.51 ± 0.745*	36.89 ± 2.29

Mean values ± SEM of the dissociation constants (Kd) and binding capacities (Bmax, fmol receptor/mg protein) in presence and absence of Gpp(NH)p are given.

*$p < 0.05$ and ***$p < 0.001$ vs. values in absence of Gpp(NH)p.

TABLE 2

ADENYLATE CYCLASE ACTIVITIES IN MEMBRANES OF WHOLE BRAINS (WITHOUT CEREBELLUM) OF RATS AFTER THEIR CONTINUOUS S.C. INFUSION WITH VEHICLE ($n = 5$) OR 1.14 μMOL FENTANYL/KG/DAY ($n = 5$) FOR 8 DAYS

	pmol cAMP/min/mg protein (25°C)	
Activity	Vehicle	Fentanyl‡
Basal	282.0 ± 10.0	254.8 ± 10.6
+ 10 μM DAMGO	270.1 ± 8.7	260.2 ± 11.3
+ 10 μM forskolin	998.9 ± 43.4***	1071.7 ± 44.8***
+ 10 μM DAMGO/ 10 μM forskolin	843.1 ± 38.6*** ‡‡‡	928.7 ± 28.9*** ‡‡‡

***$p < 0.001$ vs. basal and DAMGO and ‡‡‡$p < 0.001$ vs. forskolin (Student-Newman-Keuls post hoc test).

‡Not significant vs. vehicle for all kinds of activity.

used analgesic drug (7,16,22–25). Furthermore, fentanyl was given continuously, i.e., by a treatment schedule that is thought to lower the development of tolerance during the long-term treatment of cancer patients with morphine, compared with intermittent administration (6,12). No additional opioid was used for testing for analgesia at the completion of the infusion because this could have had a negative impact on the constant body level of drug and led to confusion over the antinociceptive effects from residual fentanyl and the probe drug.

The infusion of fentanyl by the osmotic minipumps was checked by the determination of the urinary excretion of the drug by an extraction/HPLC procedure that allowed for the very low urinary recovery of less than 1% of intact drug of a subcutaneous dose, which is in accordance with its extensive metabolism (3,13,21). From the constant values of urinary fentanyl over the experimental time from day 1 to day 8 (Fig. 1), it is concluded that a constant plasma level and, by steady-state equilibrium, constant tissue levels of the drug were maintained during the long-term infusion.

The tail-flick latencies as measured 24 (Fig. 2) and 48 h after starting the infusion of fentanyl were increased in a dose-dependent fashion. However, after 8 days of infusion, only a slight but not significant analgesic response was seen even at the highest dose used (Fig. 2). Conclusively, tolerance to the analgesic activity of fentanyl had developed at submaximal dosing of the drug and under conditions thought to be favorable in decreasing tolerance (see above).

Changes in pharmacological parameters, e.g., tolerance, of opioids during their long-term administration should be caused or accompanied by changes in the chain of molecular events following binding of the active compound to its specific receptors. Therefore, parameters of the specific receptor sites, i.e., receptor capacity and affinity, and events closely related to them, i.e., their regulation by GTP and of adenylate cyclase, has been sought as possibly being involved in the development of tolerance in laboratory animals.

Literature data generally agree that there is no change in the affinity of the μ-opioid receptors in the CNS of rats after long-term administration of μ-opioid agonists (1,2,9,15,17,27). In line with this, no significant effect of the in vivo fentanyl dose on the affinity of the μ-receptors, as measured with the μ-specific ligand DAMGO, in both the brain and spinal cord was found here (Table 1). Furthermore, the concentrations of the μ-receptors in brain and spinal cord were not altered significantly by the fentanyl treatment (Table 1), which is in line with only part of the literature data about continuous opioid administration (8,27), whereas in some reports decrease (2,9,26) or even elevation (1,5,19,20,26) in the number of binding sites were found.

μ-Opioid receptors are G(i)-protein-coupled to inhibit the adenylate cyclase. Accordingly, the GTP analogon Gpp(NH)p decreased the affinity of the DAMGO binding sites in the rat brain and spinal cord membranes (i.e., increase in the K_d by 3.09 and 2.65, respectively; Table 1) and, furthermore, DAMGO decreased the forskolin-stimulated adenylate cyclase (Table 2). However, again there was no significant influence of the long-term treatment of the rats with fentanyl on these parameters, although the analysis of the data showed a tendency to a slight inhibition of the decrease of the receptor affinity by Gpp(NH)p, as it was observed with midbrain membranes from morphine-tolerant rats (27).

The main difference between the literature studies mentioned above and this study lies in the treatment schedule of the rats with opioids. Most studies have used repeated administration of morphine, especially in pellet formulations, to obtain morphine-tolerant rats. Therefore, different and fluctuating body levels of morphine may have resulted in tolerant states of differing degrees for subsequent biochemical studies. In the present study, pharmacokinetically, by constant-rate infusion, and pharmacodynamically, by tail-flick measurements, controlled rats were used and the membrane preparations were checked to be free of contaminations of residual fentanyl.

Under these conditions, in summary, infusion of rats for 8 days with fentanyl in doses up to 1.14 μmol/kg/day, leading to tolerance to its analgesic effect, did not significantly change the capacity, affinity, and regulation of the affinity by GTP of the μ-opioid receptor in brain and spinal cord, nor did it change the activity of the brain adenylate cyclase and its inhibition by the μ-opioid ligand DAMGO. There may be two general reasons why no effects of the state of tolerance on the μ-opioid receptors in preparations of whole brain and spinal cord were observed. Molecular events in the signaling system more distant to the events at the receptor may be affected (11,14), which could give rise to additional messengers, e.g., anti-opioid peptides (18). Alternatively, or additionally, changes at the receptor level may be restricted to specific regions in the CNS thought to be mainly involved in the development of tolerance (14).

Then such site-specific changes in the receptors could hardly be seen with preparations of the whole brain. On the other hand, changes in the capacity of μ-opioid receptors have been found in a broad spectrum of brain loci such as cortex (2,26), midbrain (26), striatum (5,26), hypothalamus (5), and pons and medulla (2), however, in some cases down-regulated and in other up-regulated. If, at the same time, the receptors in different regions were regulated differently, then again using whole brain preparations would not reveal the significant processes.

It has been considered that up-regulated opioid receptors might differ from the normal inhibitory receptors by being excitatory in nature, counteracting the analgesic activity of an opioid, and that receptor up-regulation might be higher as the level of tolerance is the higher (18). This could partly explain the different results for opioid receptor regulation, assuming different levels of tolerance in the various studies. The possibility that μ-opioid receptors may be differently regulated depending on their regional localization and the level of tolerance emphasizes investigations into the regional receptor regulation under different levels of tolerance.

ACKNOWLEDGEMENTS

We thank N. Prinz, R. Schmidt, and I. Gogalla for their excellent technical assistance, and Dr. E. Friderichs from Grünenthal GmbH (Aachen, F.R.G.) for pharmacological consultation. This work was supported by the Fonds der Chemischen Industrie, Frankfurt/Main (F.R.G.).

REFERENCES

1. Besse, D.; Lombard, M. C.; Besson, J. M.: Up-regulation of (H-3)DAMGO and (H-3)DTLET opioid binding sites in laminae I-II of the spinal cord in intact and deafferented morphine-tolerant rats. Neuroscience Lett. 136:209–212; 1992.
2. Bhargava, H. N.; Gulati, A.: Down-regulation of brain and spinal cord μ-opiate receptors in morphine tolerant-dependent rats. European J. Pharmacol. 190:305–311; 1990.
3. Björkman, S.; Stanski, D. R.; Verotta, D.; Harashima, H.: Comparative tissue concentration profiles of fentanyl and alfentanil in humans predicted from tissue/blood partition data obtained in rats. Anesthesiology 72:865–873; 1990.
4. Bradford, M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254; 1976.
5. Brady, L. S.; Herkenham, M.; Long, J. B.; Rothman, R. B.: Chronic morphine increases μ-opiate receptor binding in rat brain: A quantitative autoradiographic study. Brain Res. 477: 382–386; 1989.
6. Collin, E.; Poulain, P.; Gauvainpiquard, A.; Petit, G.; Pichardleandri, E.: Is disease progression the major factor in morphine tolerance in cancer pain treatment. Pain 55:319–326; 1993.
7. Cox, B. M.; Werling, L. L.: Opioid tolerance and dependence. In: Pratt, J. A., ed. The biological bases of drug tolerance and dependence. London: Academic Press, Harcourt Brace Jovanovich, Publishers; 1991:199–229.
8. De Vries, T. J.; Ril, G. H. K. T. T.; Van der Laan, J. W.; Mulder, A. H.; Schoffelmeer, A. N. M.: Chronic exposure to morphine and naltrexone induces changes in catecholaminergic neurotransmission in rat brain without altering mu-opioid receptor sensitivity. Life Sci. 52:1685–1693; 1993.
9. Diaz, A.; Ruiz, F.; Florez, J.; Hurle, M. A.; Pazos, A.: Mu-opioid receptor regulation during opioid tolerance and supersensitivity in rat central nervous system. J. Pharmacol. Exp. Ther. 274:1545–1551; 1995.
10. Duman, R. S.; Tallman, J. F.; Nestler, E. J.: Acute and chronic opiate-regulation of adenylate cyclase in brain: Specific effects in locus coeruleus. J. Pharmacol. Exp. Ther. 246:1033–1039; 1988.
11. Guitart, X.; Nestler, E. J.: Second messenger and protein phosphorylation mechanisms underlying opiate addiction—Studies in the rat locus-coeruleus. Neurochemical Res. 18:5–13; 1993.
12. Hanks, G. W.: Pain management in cancer patients. Therapie 47: 489–493; 1992.
13. Lehmann, K. A.; Hunger, L.; Brandt, K.; Daub, D.: Biotransformation von Fentanyl. III. Einflüsse chronischer Arzneimittelexposition auf Verteilung, Metabolismus und Ausscheidung bei der Ratte. Anaesthesist 32:165–173; 1983.
14. Nestler, E. J.; Erdos, J. J.; Terwilliger, R.; Duman, R. S.; Tallman, J. F.: Regulation of G proteins by chronic morphine in the rat locus coeruleus. Brain Res. 476:230–239; 1989.
15. Nishino, K.; Su, Y. F.; Wong, C. -S.; Watkins, W. D.; Chang, K. J.: Dissociation of mu opioid tolerance from receptor down-regulation in rat spinal cord. J. Pharmacol. Exp. Ther. 253:67–72; 1990.
16. Paronis, C. A.; Holtzman, S. G.: Development of tolerance to the analgesic activity of μ-agonists after continuous infusion of morphine, meperidine or fentanyl in rats. J. Pharmacol. Exp. Ther. 262:1–9; 1992.
17. Rogers, N. F.; El-Fakahany, E. E.: Morphine-induced opioid receptor down-regulation detected in intact adult rat brain cells. European J. Pharmacol. 124:221–230; 1986.
18. Rothman, R. B.: A review of the role of anti-opioid peptides in morphine tolerance and dependence. Synapse 12:129–138; 1992.
19. Rothman, R. B.; Bykov, V.; Long, J. B.; Brady, L. S.; Jacobson, A. E.; Rice, K. C.; Holaday, J. W.: Chronic administration of morphine and naltrexone up-regulate μ-opioid binding sites labeled by [^{3}H]-D-Ala2,MePhe4,Gly-ol5-enkephalin: Further evidence for two μ-binding sites. European J. Pharmacol. 160:71–82; 1989.
20. Rothman, R. B.; McLean, S.; Bykov, V.; Lessor, R. A.; Jacobson, A. E.; Rice, K. C.; Holaday, J. W.: Chronic morphine upregulates a μ-opiate binding site labeled by [^{3}H]cycloFOXY: A novel opiate antagonist suitable for positron emission tomography. Eur. J. Pharmacol. 142:73–81; 1987.
21. Schneider, E.; Brune, K.: Distribution of fentanyl in rats: An autoradiographic study. Naunyn-Schmiedebergs Arch. Pharmacol. 331: 359–363; 1985.
22. Sosnowski, M.; Yaksh, T. L.: Differential cross-tolerance between intrathecal morphine and sufentanil in the rat. Anesthesiology 73:1141–1147; 1990.
23. Stevens, C. W.: Perspectives on opioid tolerance from basic research: Behavioural studies after spinal administration in rodents. Cancer Surveys 21:25–47; 1994.
24. Stevens, C. W.; Yaksh, T. L.: Potency of infused spinal antinociceptive agents is inversely related to magnitude of tolerance after continuous infusion. J. Pharmacol. Exp. Ther. 250:1–8; 1989a.
25. Stevens, C. W.; Yaksh, T. L.: Time course characteristics of tolerance development to continuously infused antinociceptive agents in rat spinal cord. J. Pharmacol. Exp. Ther. 251:216–223; 1989b.
26. Tao, P. -L.; Law, P. -Y.; Loh, H. H.: Decrease in delta and mu opioid receptor binding capacity in rat brain after chronic etorphine treatment. J. Pharmacol. Exp. Ther. 240:809–816; 1987.
27. Tao, P. -L.; Lee, C. -R.; Law, P. -Y.; Loh, H. H.: The interaction of the mu-opioid receptor and G protein is altered after chronic morphine treatment in rats. Naunyn-Schmiedebergs Arch. Pharmacol. 348:504–508; 1993.
28. Werling, L. L.; McMahon, P. N.; Cox, B. M.: Selective changes in μ opioid receptor properties induced by chronic morphine exposure. Proc. Natl. Acad. Sci. USA 86:6393–6397; 1989.
29. Winer, B. J.; Brown, D. R.; Michels, K. M.: Statistical principles in experimental design, 3rd edition; New York: McGraw-Hill, Inc. 1991.