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Lack of Cross-Tolerance on Multiple Opiate Receptors in the Mouse Vas Deferens

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SUMMARY

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The development of opiate tolerance has been studied in the mouse vas deferens, which contains μ- and δ-opiate receptors. Long-term infusion of opioids acting selectively on either μ- or δ-receptors was accomplished by means of osmotic minipumps. *In vitro* tests of vasa deferentia from chronically [D-Ala^2,D-Leu^5]enkephalin (DADL)-treated mice revealed an 800-fold degree of tolerance for δ-receptors, while μ-receptors were left unaffected. Accordingly, cross-tolerance to δ-receptor agonists, e.g., leucine–enkephalin, was observed but not to μ-receptor agonists, e.g., normorphine. On the other hand, infusion of the potent opioid sufentanyl, which almost selectively acts at μ-receptors, brings about a high degree of tolerance for μ-receptor agonists, but causes no change in sensitivity to δ-receptor agonists. A number of opioids, including β-endorphin, exhibit some degree of cross-tolerance in δ-receptor-tolerant as well as in μ-receptor-tolerant preparations. Essentially identical results were obtained in vasa deferentia made acutely tolerant *in vitro* using the δ-receptor agonist DADL. Challenge of highly tolerant vasa deferentia with the narcotic antagonist naloxone failed to demonstrate any withdrawal sign. The failure to demonstrate dependence in highly tolerant vasa deferentia may be due to adaptational processes upon chronic opioid exposure at the opiate receptor binding site level. It is concluded that opiate receptor differentiation can be reliably achieved by means of their selective tolerance development.

INTRODUCTION

Much evidence for the concept of multiple opiate receptors is based on studies conducted on isolated tissues (1–4). In these investigations, a differentiation of opiate receptors was achieved by determining the biological activity as well as the binding characteristics of specific opioids in preparations from naive animals.

If a multiplicity of opiate receptors implies their independent occurrence, then an essentially different approach to the classification of these receptors may be attained by means of their chronic activation, as has been proposed by Martin *et al.* (5). A prolonged, selective activation of a specific opiate receptor population should cause a circumscribed development of tolerance and dependence, confined to this specific population, leaving other opiate receptor types unaffected. Such an occurrence, however, would conflict with the well-known phenomenon of cross-tolerance postulated for opioids, that is, that the development of tolerance to one opioid is always associated with the appearance of cross-tolerance to all other narcotics (6, 7). In order to test the proposed hypothesis that a selective development of tolerance/dependence, associated with certain receptor types, can occur, a test system for these different receptor types is needed. The vas deferens of the mouse (MVD) would provide a suitable preparation on which to work, since μ- and δ-opiate receptors are inherent in this tissue (1, 8). Moreover, highly selective and potent narcotic agonists are available for these receptors, such as [D-Ala^2,D-Leu^5]enkephalin (DADL) for δ-receptors (4, 9) and sufentanyl (SUF) for μ-receptors (10), so that the essential prerequisites are fulfilled.

We have evidence that the selective activation of specific opiate receptors in the MVD results in tolerance development associated with distinct types of opiate receptors (10–12). Extending the recent findings, the present investigations put emphasis on the phenomena tolerance, cross-tolerance, and dependence in vasa deferentia of mice after prolonged exposure to specific opioids.

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METHODS

Vasa deferentia of NMRI mice (25–30 g) were prepared (13) and set up in a 5-ml organ bath (3) for electrical stimulation to induce a maximal twitch tension (60 V, 0.1 Hz, 0.5 ms; resting tension, 50 mg). The composition (mM) of the Krebs–Ringer solution was NaCl, 118; KCl, 4.75; $CaCl_2$, 2.54; KH_2PO_4, 1.19; glucose, 11.0; $NaHCO_3$, 25.0; l-tyrosine, 0.25. The preparations were equilibrated for at least 30 min by changing the incubation medium at 5- to 10-min intervals. The drug effects on twitch tension are expressed as percentages of the tension of the electrically induced twitches just before the drug was added to the bath. The drug concentration causing a 50% inhibition of twitch tension is called IC_{50}.

Osmotic minipumps (Alza Corp., Palo Alto, Calif., Model 2001) were employed for chronic drug administration, having a delivery rate of 1 μl/h. The pumps were implanted subcutaneously under light ether anesthesia. Drug supply was terminated by removal of the pumps. The serum concentrations of opioids of infused mice were assayed by testing the serum directly on the electrically stimulated MVD preparation, using the opioid to be determined as a standard (14). Serum of naive mice did not interfere with electrically evoked twitch tension.

The following substances were used: naloxone (Endo Laboratories, Inc.), normorphine (Boehringer, Ingelheim), etorphine (Reckitt and Colman), [D-Ala^2,D-Leu^5]enkephalin (Bachem), methionine–enkephalin (Met–enkephalin), leucine–enkephalin (Leu–enkephalin), l-tyrosine (Serva), sufentanyl (Janssen Pharmaceutica), FK 33-824 (Sandoz), and β_h-lipotropin$_{61-91}$ (Peninsula Labs). β_h-Lipotropin$_{61-79}$ was donated by Dr. Ling (The Salk Institute). The drug concentrations employed refer to their free base.

RESULTS

Tolerance development to δ-receptor agonists. Figure 1 demonstrates the opioid activity of DADL on vasa deferentia from naive mice and from animals chronically infused with 5 μg DADL/h for 1, 3, or 6 days. To prevent freeing of opiate receptors from agonist *in vitro*, the preparations of chronically DADL-infused mice were exposed to 20 nM DADL, which was the mean concentration measured in the serum at different times during the course of infusion and at killing, as soon as the tissue was removed from the animal and kept continuously in contact with it during the following procedure. The IC_{50} for DADL determined in preparations of naive animals amount to 0.36 ± 0.004 nM, while the dose–response curves shift to the right in vasa deferentia from chronically DADL-treated mice. In the presence of 20 nM DADL the IC_{50} was 65 ± 8 nM in preparations from mice infused for 1 day, 105 ± 11 nM after 3 days of infusion, and 290 ± 24 nM after 6 days. Extending the infusion for 9 days did not further increase the IC_{50}. Thus, 6 days of DADL (5 μg/h) treatment caused a high degree of tolerance (800-fold) to this enkephalin derivative. For data regarding the electrically evoked twitch tension of DADL-tolerant preparations, see the following.

Termination of DADL incubation *in vitro* was caused by exposing the preparations to Krebs–Ringer solution lacking DADL. The bathing fluid was renewed at 5- to 10-min intervals for 3 h. Thereafter the dose–response curves shifted to the left, indicating recovery of sensitivity (Fig. 1, dashed lines). Those preparations least exposed *in vivo* to DADL showed more effective recovery after terminating DADL exposure *in vitro*. The respective IC_{50} values are 1.5 ± 0.02 nM (1-day infusion of mice and 3-h wash *in vitro*), 36 ± 4 nM (3 days), and 65 ± 7 nM (6 days). A further 2-h wash of these preparations did not significantly reduce the IC_{50} values.

The high degree of DADL tolerance (5 μg/h, 6 days) appears to be reversible (Fig. 2). Drug supply was terminated by removing the pumps, and the vasa deferentia were dissected out after certain periods and set up *in vitro* in the absence of any drug. Six hours after termi-

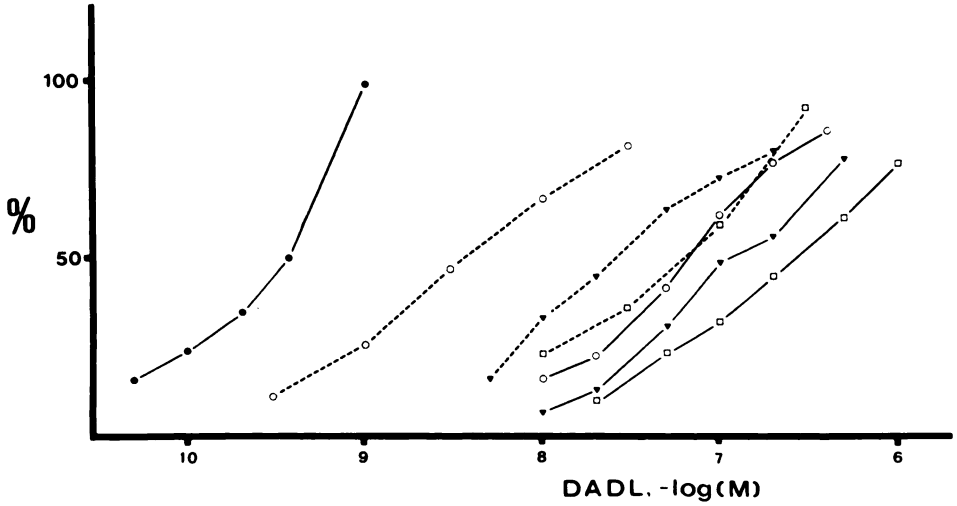

FIG. 1. *Dose–response curves for DADL on electrically stimulated vasa deferentia of naive mice* (●) *and of preparations isolated from DADL-infused mice (5 μg/h) for 1 day* (○), *3 days* (▼), *and 6 days* (□)

The chronically DADL-exposed preparations were kept at 20 nM DADL *in vitro*, a concentration measured in the plasma at the time of killing. The dashed lines reflect dose–response curves obtained after washing the preparation in the absence of DADL for 3 h. Ordinate: percentage of twitch tension of the preceding electrically induced twitches. Abscissa: DADL concentration (M). Each point is the mean of eight tests on tissues from different animals. The standard error of the mean of each point did not exceed 12%.

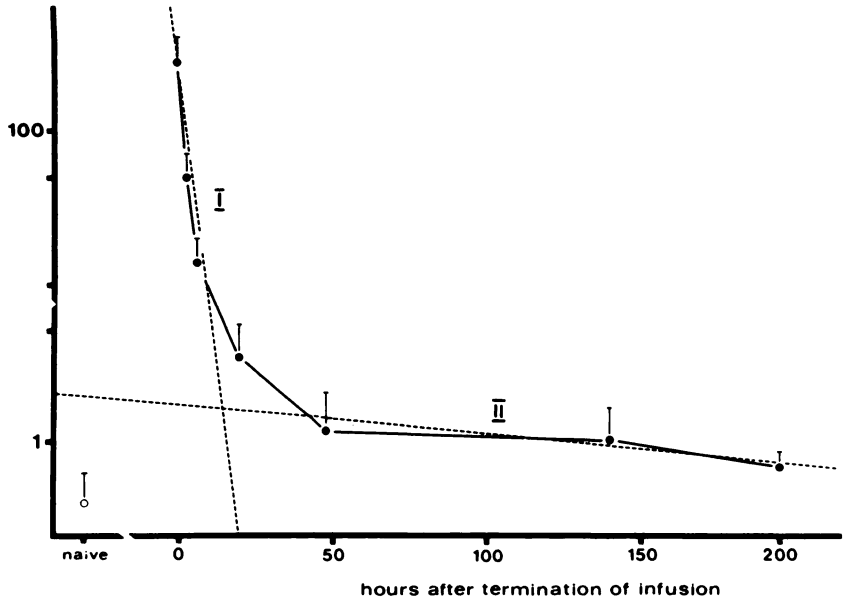

FIG. 2. *IC_{50} values for DADL on vasa deferentia of naive mice* (○) *and of mice infused with DADL (5 μg/h, 6 days), but killed at different times after termination of the drug supply* (●)

Each point reflects the mean IC_{50} value. Vertical bars indicate standard errors. The single data were read off from at least six dose–response curves performed on tissues of different animals. Ordinate: IC_{50} values (nM). Abscissa: time (h) after termination of DADL supply. Dashed lines indicate two components of recovery, having a half-life (I) of 2 h and (II) of 163 h.

nation of DADL supply, the serum concentration of this opioid was below the detection limit (2 nM), and a considerable decrease in tolerance was observed, as indicated by an IC_{50} value of only 14 ± 3 nM. Thereafter, a much slower decline occurred, showing an IC_{50} of 0.7 ± 0.36 nM at 192 h.

The availability of δ-receptor-tolerant vasa deferentia led to tests of a number of opioids which have been described as having either μ- or δ-receptor activity (1, 4, 9). Table 1 presents the IC_{50} values obtained from DADL-tolerant vasa deferentia and from naive preparations. Obviously, Leu–enkephalin shows the highest degree of cross-tolerance in DADL-tolerant preparations (Q = 252), while etorphine, normorphine, and dihydromorphine exhibited no cross-tolerance at all. Some cross-tolerance was displayed to different fragments of β-LPH, and only a moderate degree was shown toward sufentanyl, ketocyclazocine, and FK 33-824.

Tolerance development to μ-receptor agonists. The failure of some opioids to exhibit cross-tolerance in DADL-tolerant preparations led to experiments to test whether chronic exposure of mice to those presumed μ-receptor agonists would bring about tolerance confined to μ-receptors. Two potent opiates on DADL-tolerant preparations, that is, etorphine and sufentanyl, were selected to test this hypothesis.

Figure 3 shows dose–response curves for etorphine (Fig. 3A) and DADL (Fig. 3B) on preparations from untreated and chronically etorphine-infused mice. Preparations of mice infused with 1 μg etorphine/h for 6 days were set up *in vitro* at 1 nM etorphine, the respective serum concentration, and those receiving 5 μg/h (6 days) were incubated at 5 nM. It appears that chronic etorphine treatment dose-dependently shifts the dose–response curve for etorphine to the right. On the other hand, the δ-receptor agonist DADL fails to show cross-tolerance in preparations from mice infused with 1 μg etorphine/h, although when 5 μg etorphine/h was infused, the activity of DADL was affected, as indicated by the 10-fold shift to the right of the dose–response curve (Fig. 3B). The electrically evoked twitch tension of vasa deferentia in the presence of 1 and 5 nM etorphine was about 80% of that measured in naive preparations (see the following).

Different results were obtained with vasa deferentia of mice infused with SUF (10 μg/h) for 6 days, which were more than 1000-fold tolerant to SUF, but which exhibited almost no change in sensitivity to DADL (11).

Table 2 summarizes the activity (IC_{50} values) of a number of opioids in SUF-tolerant preparations as compared to that in naive vasa deferentia. Apparently, com-

TABLE 1

Opioid sensitivity of DADL-tolerant MVD[a] (IC_{50}, nM)[b]

Compound	Chronic DADL exposure	Naive	Q, chronic/naive
DADL	290 ± 24	0.36 ± 0.04	806
Leu–enkephalin	3020 ± 350	12 ± 1.5	252
β-LPH$_{61-89}$	580 ± 60	11 ± 1.2	53
β-LPH$_{61-79}$	1670 ± 140	35 ± 3.9	48
Met–enkephalin	660 ± 58	26 ± 2.8	25
β-Endorphin	1900 ± 200	118 ± 12	16
Sufentanyl	0.2 ± 0.01	0.04 ± 0.005	5
FK 33-824	30 ± 4	14 ± 1.6	2
Ketocyclazocine	50 ± 8	31 ± 2.9	1.6
Etorphine	0.54 ± 0.06	0.5 ± 0.06	1.1
Normorphine	750 ± 79	750 ± 68	1
Dihydromorphine	220 ± 23	210 ± 19	1

[a] Infusion of 5 μg DADL/h for 6 days, preparations set up under 20 nM DADL.

[b] Each value (mean ± SE) consists of at least eight determinations on preparations from different mice.

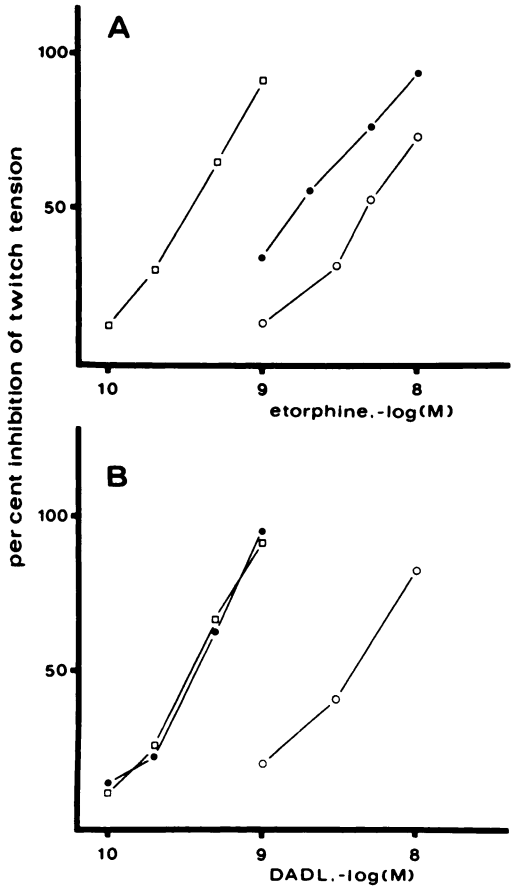

FIG. 3. *Dose–response curves for etorphine (A) and DADL (B) on vasa deferentia from untreated mice (□) and from mice infused with 1 μg etorphine/h (●) or 5 μg/h (○) each for 6 days*

The preparations were set up *in vitro* either in the absence of any drug (□) or at the etorphine concentration determined in the plasma at the time of killing of the mice (1 μg/h, 2 nM; 5 μg/h, 5 nM). Each point represents the mean of at least eight determinations on tissues from different mice. The standard error of each point did not exceed 15%. Abscissa: molarity of drug concentration. Ordinate: percentage inhibition of electrically stimulated twitch tension.

pounds lacking cross-tolerance in DADL-tolerant preparations show a high degree of tolerance in SUF-tolerant tissues. Conversely, presumed δ-agonists, such as DADL and Leu-enkephalin, lack cross-tolerance in SUF-tolerant preparations. The other compounds tested showed a moderate degree of cross-tolerance.

Besides the findings reported for the vasa deferentia, the effect of SUF on the urinary bladder, as compared to the action of DADL, is of particular interest. Mice which were chronically exposed to SUF (10 μg/h) exhibited a spasm of the vesical sphincter after even 9 days of treatment. Obviously, this chronic action of SUF was not accompanied by development of tolerance. In contrast, we never observed a spasm of the vesical sphincter during chronic infusion of DADL.

Combined administration of δ- and μ-receptor agonists. Mice were simultaneously infused with DADL (5 μg/h) and SUF (10 μg/h) for 6 days. Their vasa deferentia were set up in the presence of their respective serum opioid concentrations. Such preparations exhibited an extremely high degree of tolerance to DADL, SUF, etorphine, Met-enkephalin, Leu-enkephalin, β-endorphin, and FK 33-824. Exposure to high concentrations of each compound (10^{-6} M) caused an inhibition of twitch tension up to only 20%. However, ketocyclazocine had an IC_{50} in those preparations of 65 nM, which is only 2.1-fold more than required for naive preparations.

Naloxone challenge of tolerant MVD. Figure 4 compares the electrically evoked twitch tension of preparations from naive mice (96 mg) with that of DADL-tolerant vasa deferentia (5 μg DADL/h, infused for 1, 3, or 6 days) set up at 20 nM DADL. Vasa deferentia of mice infused with DADL for 1 day develop a tension of 70% ($P < 0.01$, $N = 18$) compared to naive controls. However, after 3 or 6 days of DADL exposure, the electrically evoked tension in the presence of 20 nM DADL was only 5 to 10% below that of controls. Naloxone challenge (300 nM) of tolerant preparations in the presence of 20 nM DADL did not change the resting tension (absence of electrical stimulation of any preparation). Naloxone given during electrical stimulation increased the tension moderately to normal values in preparations of mice infused for 1, 3, or 6 days, that is, no withdrawal sign was detectable. The failure to exhibit an increased tension appears not to be due to a general inability of the preparations to stronger contract, since those preparations responded to serotonin (100–500 nM) with a further increase in twitch tension. Identical results were obtained employing submaximal electrical stimulation (20 V).

Somewhat different results were obtained with SUF-infused mice (10 μg/h, 6 days). Vasa deferentia set up at 5 nM SUF responded to electrical stimulation with a wide range of twitch tensions. About 20% of all the preparations developed tensions below 50% of that of naive MVD in the absence of any drug. Such vasa deferentia were discarded. The other SUF-tolerant preparations exhibited tensions of up to 80% that of naive tissues. Challenge of those preparations with naloxone (300 nM) failed to affect the resting tension, but increased electrically evoked twitch tension by 30% above that of normal preparations. The augmented twitch tension declined in the presence of the narcotic antagonist if no washes were

TABLE 2

Cross-tolerance of sufentanyl-tolerant MVD[a] (IC_{50}, nM)[b]

Compound	Chronic SUF exposure	Naive	Q, chronic/naive
Sufentanyl	50 ± 6	0.04 ± 0.005	1250
Dihydromorphine	23,000 ± 1,800	210 ± 19	110
Normorphine	70,000 ± 5,000	750 ± 68	93
FK 33-824	370 ± 41	14 ± 1.6	26
Etorphine	2.1 ± 0.3	0.54 ± 0.006	3.8
β-Endorphin	542 ± 48	118 ± 12	4.6
Met-enkephalin	73 ± 6	26 ± 2.8	2.8
Ketocyclazocine	56 ± 5	31 ± 2.9	1.8
Leu-enkephalin	10	12 ± 1.5	0.8
β-LPH$_{61-69}$	13 ± 1.4	11 ± 1.2	1.2
DADL	0.4	0.36 ± 0.04	1.1

[a] Infusion of 10 μg sufentanyl/h for 6 days, preparations set up under 5 nM sufentanyl.

[b] Each value (mean ± SE) consists of at least six determinations on preparations from different mice.

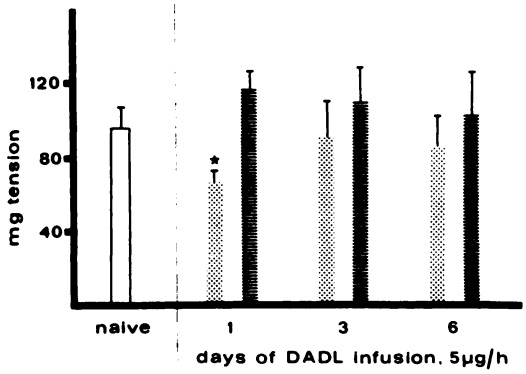

FIG. 4. *Electrically induced twitch tension of vasa deferentia from untreated mice (open column) and from those infused with DADL (5 µg/h) for 1, 3, and 6 days*

The preparations from chronically treated mice were set up under 20 nM DADL (stippled columns). Naloxone challenge (200 nM) was conducted in the presence of the opioid (hatched columns). Columns represent means of at least 12 preparations ± SE. $^*P < 0.01$ (as compared to naive preparations).

applied. However, naive control preparations also lose tension to a similar degree if no washes are applied. If preparations were exposed *in vitro* to 0.1 nM SUF (that is, 10-fold below the serum concentration in mice from which the vasa deferentia were taken), the degree of tolerance to SUF was only 200-fold. However, under such conditions the tension was not different from that of control preparations and naloxone completely failed to affect electrically evoked twitch tension.

Acute tolerance (tachyphylaxis). Vasa deferentia of naive mice were set up for electrical stimulation and washed for 1 h before incubating the preparations with different concentrations of DADL. Each concentration selected (1, 10, and 100 nM) completely eliminated the response to electrical stimulation. However, the twitch tension slowly recovered in the presence of DADL, reaching a maximum after 3 h (Table 3). This indicates the development of acute tolerance. Under these conditions the preparations displayed different degrees of sensitivity to DADL (Table 3). Washing the acutely tolerant preparations for 1 h (changes of the bath fluid at 5- to 10-min intervals) caused complete recovery of sensitivity to DADL of all tissues.

Preparations exposed for 3 h to 10 nM DADL were used to test the opioid effect of Leu-enkephalin, Met-enkephalin, β-endorphin, sufentanyl, and etorphine. In principle, the quotients calculated from the IC_{50} values obtained from acutely tolerant and naive preparations for the different compounds follow essentially the same rank order described in Table 1 for chronically DADL-tolerant preparations. Interestingly, exposure of naive preparations *in vitro* to DADL immediately after dissection caused a much faster development of acute tolerance.

Attempts failed to clearly demonstrate acute tolerance employing SUF. Preparations ($N = 10$) were exposed to 1 and 10 nM, respectively, which eliminated the response to electrical stimulation. Incubation for up to 5 h did not cause recovery as indicated by the continued lack of induction of electrically evoked twitch tension. However, naloxone challenge (500 nM) immediately brought back the twitch tension.

DISCUSSION

The MVD has been said to contain δ- and μ-opiate receptors, as characterized by specific opioids (1, 4, 8). This view is strongly supported by the demonstration of selective tolerance development of these types of opiate receptors.

Chronic δ-receptor activation by the highly specific δ-agonist DADL (4, 9) shifts the DADL dose–response curve by almost 3 orders of magnitude to the right. Compounds known to bring about their action preferentially via δ-receptors, such as Leu-enkephalin (1, 4), are expected to exhibit a high degree of cross-tolerance in such preparations. Indeed, Leu-enkephalin was 250-fold less potent in DADL-tolerant vasa deferentia. The phenomenon that Leu-enkephalin at high concentrations still displays activity in preparations extremely tolerant to the δ-receptor agonist DADL would suggest an activity via a different population of opiate receptors, e.g., μ-receptors. In fact, presumed μ-receptor agonists (FK 33-824, normorphine, dihydromorphine, SUF) are of almost identical activity in δ-receptor-tolerant preparations. Thus, these opioids seem to act selectively at μ-receptors, which are clearly not affected during chronic exposure to DADL. However, an unchanged activity of an opioid, such as etorphine, in the δ-receptor-tolerant MVD does not necessarily signal a high selectivity for μ-receptors. This becomes even more clear in mice chronically infused with etorphine, since the vas deferens of such animals developed tolerance to both μ- and δ-agonists (see Fig. 3). Thus, the powerful opiate etorphine exhibits a low selectivity for a specific type of opiate receptor, which is in line with findings, reported by others, obtained from binding studies with brain homogenate (15).

In contrast to the findings with etorphine, SUF proved a rather selective μ-receptor agonist. In analogy to chronic DADL treatment of mice, prolonged SUF infusion results in a highly SUF-tolerant vas deferens, in which DADL, β-LPH_{61-69}, as well as Leu-enkephalin exhibit no cross-tolerance at all. These findings are in line with a report by Cox (16) indicating an unchanged potency of Leu-enkephalin in the vasa deferentia of mice chronically exposed to morphine. In contrast, normorphine and dihydromorphine prove their high μ-receptor selectivity by a considerable degree of cross-tolerance. The rather low ability of morphine to cause tolerance in the MVD (19–21) appears to be due to the weak potency of morphine in the MVD (IC_{50}, 600 nM; that is, 15.000-fold less potent than sufentanyl).

TABLE 3

Acute tolerance development to DADL[a] in vitro

DADL incubation (nM)	0	1	10	100
Twitch tension[b]	1.00	0.40	0.55	0.12
IC_{50} for DADL (nM)	0.36	1.8	20	120

[a] Bath fluid changed at 15-min intervals for 3 h.

[b] Relative values; 1.00 = twitch tension in the absence of DADL.

The ability of specific opioids to still display activity in either δ-receptor-tolerant or μ-receptor-tolerant preparations suggested an action of these compounds via opiate receptors unaffected in their function. Apparently SUF represents an opiate preferentially acting on μ-receptors with moderate action on δ-receptors (see Table 1). At high concentrations, however, SUF exhibits an overlapping on other opiate receptors. This view is strongly supported by experiments with preparations from mice simultaneously infused with DADL and SUF, thus "eliminating" μ- and δ-receptors. All opioids tested, except the κ-receptor agonist ketocyclazocine, almost completely lost their activity in vasa deferentia of such animals. The almost unchanged activity of ketocyclazocine in DADL/SUF-tolerant preparations indicates the presence of κ-receptors in the MVD.

A MVD highly tolerant to DADL does recover after termination of the opioid supply. The time course required *in vivo* for regain of a normal sensitivity primarily depends on the length of the period of exposure to the agonist. The longer the vasa deferentia were in contact with the opioid, the slower was the recovery. After termination of opioid supply following 6 days of infusion, recovery of normal sensitivity requires about 1 week. The time course of recovery is biphasic, having a rapid increase of sensitivity during the first hours after removal of the opioid, followed by a much longer period of very slow recovery. In this it resembles findings reported for the morphine-tolerant myenteric plexus of the guinea pig (16).

Tests for dependence in tolerant preparations were conducted by naloxone challenge in the presence of the opioid. This procedure has been reported to reliably cause a withdrawal sign in the tolerant myenteric plexus of the guinea pig ileum, as indicated by the appearance of increased tension, resulting from the sudden termination of the narcotic action (17). Under the present conditions, highly DADL-tolerant vasa deferentia failed to exhibit any withdrawal sign upon naloxone challenge, confirming earlier reports (11). With respect to sufentanyl-tolerant preparations, it is highly questionable that the observed naloxone-induced increase in electrically evoked twitch tension above normal values actually reflects a withdrawal sign. The twitch tension before naloxone challenge was already below normal values, and the sudden disinhibition by naloxone may cause an augmented release of an excitatory neurotransmitter followed by an overshooting twitch tension. This phenomenon is known from preparations acutely exposed to morphine (18). The view that naloxone fails to cause a withdrawal sign becomes more clear in SUF-tolerant preparations exposed *in vitro* to 0.1 nM SUF only. These preparations were 200-fold tolerant, but lacked any response to naloxone. Therefore, the interpretation of former reports (19, 20) which described the naloxone response as a withdrawal sign in morphine-tolerant vasa deferentia needs to be reconsidered. This would be in line with related reports (21). Nevertheless, the inability to demonstrate a specific phenomenon does not imply its absence.

If tolerance and dependence are in fact dissociated in the MVD, it could be explained by tolerance development at the level of the opiate binding site. In fact, we have indications that DADL-tolerant vasa deferentia show an inability to specifically bind [^{3}H]DADL as compared to naive preparations (unpublished observation). A lack of opiate receptors could explain the existence of tolerance in the absence of dependence. A similar phenomenon of reduction of opiate receptors upon chronic exposure to an opiate has been described recently for certain areas of the brain (22), although most investigations deny such a mechanism of tolerance development (23).

Tolerance development of a selective opiate receptor population does not require long-term administration of the specific opioid. Acute tolerance to DADL has been documented *in vitro* after exposure of the MVD to DADL for a few hours only, while the μ-receptors are left unaffected. That is, δ-receptor agonists display a high degree of cross-tolerance, while μ-receptor agonists do not. The failure to demonstrate acute tolerance during sufentanyl incubation does not necessarily imply an inability of the MVD to develop tachyphylaxis to this μ-receptor agonist. This issue is presently under further investigation. The concept of opiate receptor differentiation by their selective tolerance development (11, 12, 24) also proved useful in studies conducted in the central nervous system (25), which revealed essentially the same results as described for the development of acute tolerance of peripherally located opiate receptors.

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