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Differentiation of Opiate Receptors in the Brain by the Selective Development of Tolerance

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SCHULZ, R., M. WÜSTER AND A. HERZ. *Differentiation of opiate receptors in the brain by the selective development of tolerance.* PHARMAC. BIOCHEM. BEHAV. 14(1) 75–79, 1981.—The selective development to either the δ-opiate receptor agonist D-Ala^2,D-Leu^5-enkephalin (DADL) or the μ-agonist sufentanyl (SUF) has been studied in the central nervous system of rats by use of osmotic minipumps for chronic administration of the drugs. The opiate-sensitive parameters analgesia and catatonia were investigated. Chronic intracerebroventricular infusion of DADL for 7 days produced a 15-fold shift in that for catatonia. In these rats, the potency of SUF in inducing analgesia and catatonia did not differ between DADL-treated animals and saline-infused controls. Similarly, chronic infusion of SUF resulted in tolerance towards SUF for both analgesia and catatonia. In these animals, DADL displayed a similar degree of tolerance w.r.t. its ability to evoke analgesia, whilst no tolerance could be detected for DADL-induced catatonia. The data indicate that prolonged stimulation of specific opiate receptors in the brain by selective agonists may bring about the selective development of tolerance for particular receptors. The data conflict with the notion that μ-receptors specifically mediate analgesia and δ-receptors catatonia.

Multiple opiate receptors Analgesia Catatonia Tolerance Cross-tolerance

A SUBSTANTIAL body of pharmacological and biochemical evidence has accumulated in support of the concept of multiple opiate receptors [2, 3, 4, 7, 15, 24]. One of the most convincing demonstration of the validity of this concept was the observation that chronic activation of particular opiate receptors in the mouse vas deferens (MVD) renders these receptors selectively tolerant to their specific agonist [19]. For example, isolated vasa deferentia of mice chronically treated with the specific δ-opiate receptor agonist D-Ala^2,Leu^5-enkephalin (DADL) are 3 orders of magnitude less sensitive to this specific agonist, whereas no cross-tolerance exists to μ-receptor agonists. This finding raises the possibility that differentiation of opiate receptors in the central nervous system (CNS) could also be achieved by the selective development of tolerance to these.

An examination of the development of tolerance in the CNS by pharmacological methods would require the use of easily evaluated opiate-sensitive parameters, such as analgesia or catatonia. Use of these parameters would seem to be advantageous, since analgesia has been suggested to be mediated by μ-receptors [3, 11, 14, 18, 23] and behavioural phenomena, which may include catatonia, by δ-receptors [4,22]. Thus, chronic activation of μ- and δ-receptors by their selective agonists, that is, sufentanyl (SUF) and DADL [10, 24, 25], respectively, could provide the basis to study differentiation and function of opiate receptors in the CNS.

METHOD

Male Sprague Dawley rats (250 g) were used throughout. For administration of the opioid peptide DADL, guide cannulas were inserted into the left and/or right lateral ventricles (for technical details see [13]). One of the guide cannulas was used for the chronic infusion, whilst the second was required for the acute tests with DADL. The peptide was injected within 5 sec in a total volume of 10 μl saline. At the end of the experiment, 10 μl of 1% trypan blue solution was injected through each cannula. Immediately thereafter the rats were decapitated to examine the placement of the cannulas. Data were evaluated only from those animal in which the spread of the dye indicated a correct injection site.

Chronic administration of drugs was accomplished by osmotic minipumps (Model 2001, Alza Corp., Palo Alto), possessing a delivery rate of 1 μl/hr for 9 days. These devices were implanted subcutaneously in order to deliver the drug either at their site of location or, via a connecting tubing to a cannula placed within the intraventricularly implanted guide cannula, directly into the CNS. The infusion of SUF in doses above 0.5 μg/hr caused death in some animals. In order to overcome the problem of the danger of initial period of drug infusion, all rats simultaneously received 1 mg/kg naltrexone (SC). As the narcotic antagonist is eliminated, the opiate receptors became occupied by SUF during the course of infusion. The use of this technique prevented the death of the

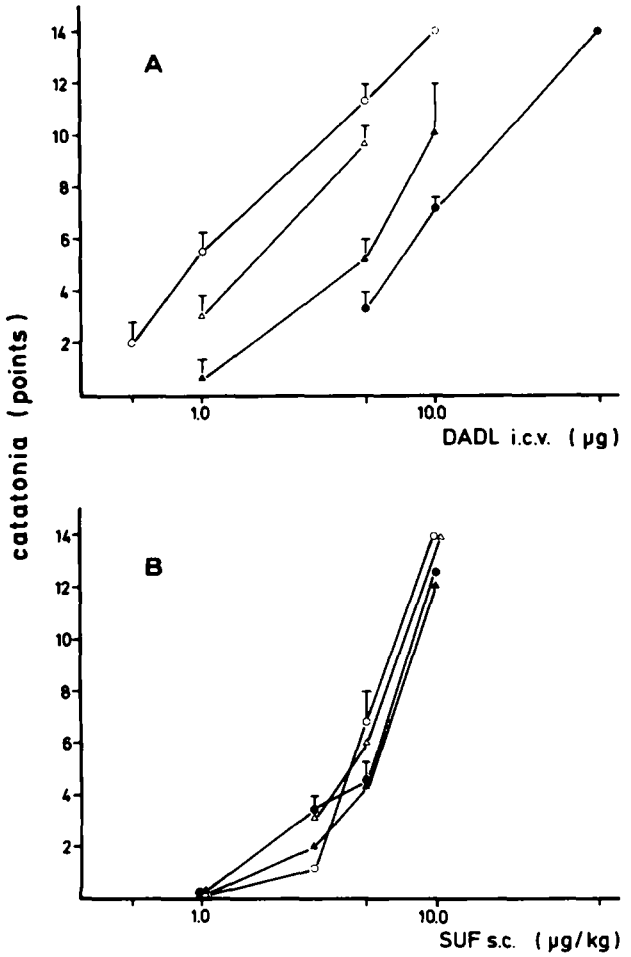

FIG. 1. Dose-response curves for the catatonic effect of D-Ala²,D-Leu⁵-enkephalin (DADL) (A) and sufentanyl (SUF) (B) in control rats (o, saline-infused) and rats chronically infused with 5 µg DADL per hour (△), 10 µg/hr (▲) and 50 µg/hr (●) for 7 or 8 days. A, abscissa: test dose of DADL (µg) given ICV per animal. B, abscissa: test dose of SUF (µg/kg) given SC. Ordinate: score for catatonia. Vertical bars indicate standard error of the mean. Each value consists of 6–10 tests on different animals. For the purpose of clarity, each standard error is not given, these were in no case greater than 16%.

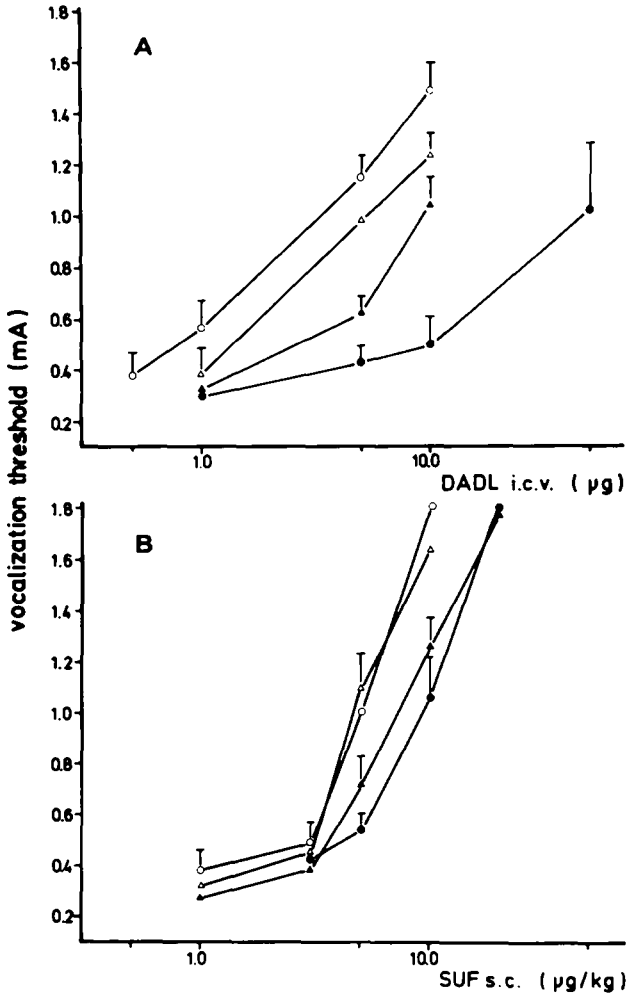

FIG. 2. Dose-response curves for the analgetic effect of DADL and SUF, respectively, on control rats (○) and those chronically infused with DADL. For further explanations see Fig. 1. The animals tested are the same as those employed for the evaluation of the catatonic action of DADL and SUF as presented in Fig. 1.

rats. The tests for analgesia and catatonia were conducted with the same animals on, respectively, the days 7 and 8 after commencement of infusion. This period of infusion proved sufficient to cause a high degree of tolerance on opiate receptors in the MVD [19]. Control rats were infused for a corresponding time with saline.

The "vocalization test" [8] was employed for quantification of analgesia. Vocalization was evoked by electrical stimulation (10 msec pulses of 50 Hz, delivered for 2 sec) of the root of the tail, employing a bipolar clamp electrode. The stimulus intensity (mA) required for vocalization was used as an indication of analgesia. In order to quantify the degree of tolerance, the 1 mA threshold was defined as EC_{50}, which reflects an about 3-fold increase in threshold.

The degree of catatonia was evaluated according to the method of Kostowski and Członkowski [12]. In general, points were scored if the rat maintained an abnormal position of the frontal or hind paw, which had been placed on blocks of 3 or 6 cm height, for more than 15 sec. The maximum score obtainable was 14 points. Introduction of a half maximal catatonic effect (7 points) has been termed also EC_{50}.

Each rat was examined for its basal vocalization threshold, before any tests were conducted. The maximal analgesic and catatonic effect of SUF was observed 5 min after subcutaneous administration. With respect to DADL given intracerebroventricularly (ICV), maximal effects were measured after 15 min.

The drugs employed are: sufentanyl (Janssen, Beerse, Belgium), D-Ala²,D-Leu⁵-enkephalin (Bachem, Basle, Switzerland), naltrexone HCl, naloxone HCl (Endo Laboratories, Garden City, New York).

RESULT

The data presented were obtained from both naive rats and those chronically treated with an opioid. All long-term treated animals neither exhibited a state of catatonia (score zero) nor responded in the vocalization threshold test differ-

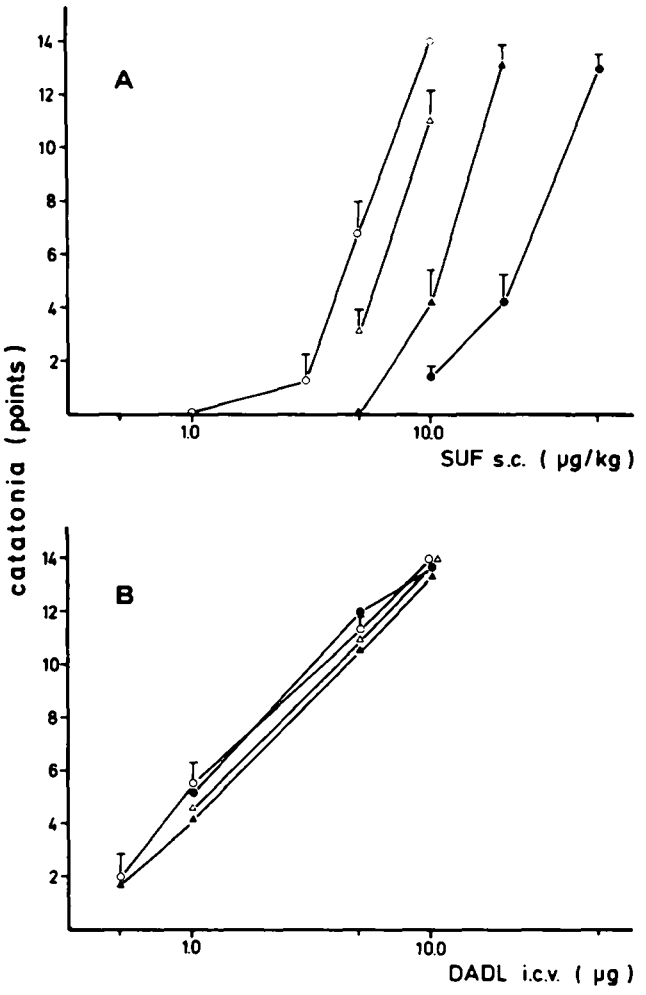

FIG. 3. Dose-response curves for the catatonic effect of SUF and DADL in control rats (○) and rats chronically infused with 0.5 µg SUF per hour (△), 1.0 µg/hr (▲) and 2.0 µg/hr (●). For further explanations see Fig. 1.

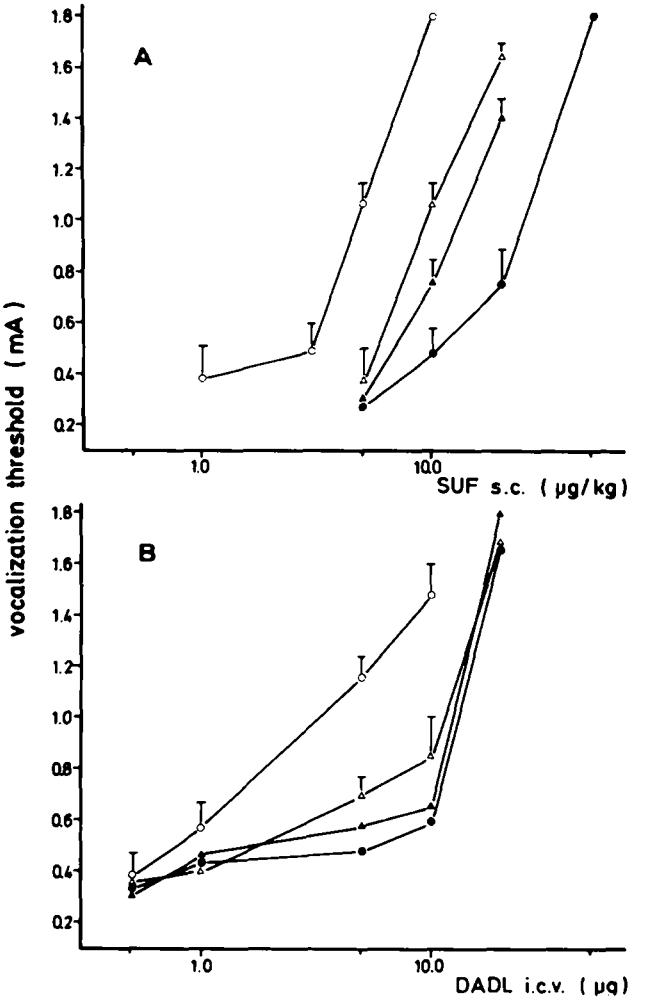

FIG. 4. Dose-response curves for the analgesic effect of SUF and DADL in control rats (○) and rats chronically infused with 0.5 µg/hr (△), 1.0 µg/hr (▲) and 2.0 µg/hr (●). For further explanations see Fig. 1. Each value consists of at least 10 tests from different animals.

ently from untreated controls (controls: 0.346 ± 0.016 mA, n=14; chronically SUF-exposed: 0.353 ± 0.011 mA, n=12; chronically DADL-exposed: 0.349 ± 0.013 mA, n=16).

Figure 1A demonstrates that the dose-response curve for DADL-induced catatonia is progressively shifted to the right as the dose of DADL infused ICV per hour is increased. At the highest dose employed, the EC_{50} value increased 6.7-fold. In contrast, there was almost no shift in the dose-response curves for SUF-evoked catatonia in these DADL-tolerant animals (Fig. 1B). The same rats were employed for testing analgesia. Tolerance to DADL is apparent, as revealed by a 15-fold shift of the dose-response curve in rats with the maximal dose of infused DADL (Fig. 2A). These animals, however, show some degree of cross-tolerance to SUF, as double the dose, as compared to controls, of the µ-agonist SUF is required in DADL-exposed (50 µg/hr) rats in order to evoke the same degree of analgesia (Fig. 2B). It should be noted that the analgesic and catatonic effect of the highest dose of DADL tested in naive animals (50 µg per rat, ICV) was completely antagonized by 1 mg/kg naloxone (SC).

In analogy to the test conducted with rats chronically infused with DADL, the additional experiments concern the effect of chronic SUF infusion upon the catatonic and analgesic potencies of SUF and DADL. Apparently, the dose-response curves for SUF-evoked catatonia shift to the right as the dose infused per hour is increased (Fig. 3A). The EC_{50} value increased 5-fold at the highest dose of SUF chronically applied (2 µg/hr). Evidently, these animals failed to display any tolerance to the catatonic effect of DADL (Fig. 3B). With respect to the analgesic effect of SUF (EC_{50}), these rats revealed a 5-fold tolerance to this µ-agonist (Fig. 4A). Interestingly, the same animals also displayed tolerance to the analgesic action of DADL. Figure 4B reveals, however, that the dose-response curves—each value of the curves represents the mean of at least 10 tests—shift to the right in a non-parallel fashion. The degree of tolerance displayed was 3.5-fold at the highest SUF dose infused (2 µg/hr), as estimated by a comparison of EC_{50} values. This changes considerably, however, when the calculations are based on the 0.5 mA (7-fold) or 1.5 mA (2-fold) vocalization threshold.

DISCUSSION

The findings reported herein demonstrate that a differentiation of opiate receptors within the CNS may be

achieved by means of the selective development of tolerance by those technique documented as reliable for the study of peripherally located opiate receptors [19,25]. Although the experimental approach employed here differs from that employed by Gilbert and Martin [7], the outcome of both investigations—based upon the development of tolerance by opiate receptors—strongly supports the concept of the existence of multiple opiate receptors.

The two opiate-sensitive parameters employed, that is, catatonia and analgesia, revealed differing pattern of results with the development of tolerance and cross-tolerance. With respect to catatonia, a clear cut result was observed: chronic exposure to DADL resulted in tolerance to the action of this δ-receptor agonist, whereas the μ-agonist SUF did not display cross-tolerance in these animals. Correspondingly, rats rendered tolerant to the catatonic effect of SUF failed to exhibit cross-tolerance to DADL. Less clear as concerns opiate receptors differentiation were the results obtained from experiments involving measurement of the analgesic action of the opioids. Chronic exposure to DADL resulted in the development of a 15-fold tolerance to this enkephalin derivative, yet μ-receptors also developed tolerance, although to a much lower degree (2-fold as measured by the response to SUF). The least selective of the manipulation proved to be the chronic infusion of SUF, since the dose-response curves for analgesia were shifted for SUF as well as for DADL in rats thus treated. These findings clearly demonstrate that an agonist loses its selective action for a particular receptor population as the dose infused increases.

A striking difference is apparent in comparing the effect of chronic exposure to either μ- or δ-receptor agonists upon peripherally and centrally located opiate receptors. Whilst in the mouse vas deferens a coexistence of highly tolerant δ-receptors (1000-fold) with μ-receptors of normal sensitivity [19] is found, the data obtained in this respect in the CNS reveal a far less dramatic distinction. The maximal degree of selective development of tolerance was only 15-fold, which does not represent a high degree of tolerance. Indeed, preliminary data concerning the degree of physical dependence shown by these animals reveal that naloxone precipitation of withdrawal fails to elicit jumping, which represents the most prominent sign of opiate withdrawal [1]. With respect to the data obtained by use of the vocalization test it appears possible that the rather low sensitivity of this technique precludes the detection of differences of a lesser magnitude.

There is no simple explanation for the considerable discrepancy observed in the response of both peripherally and centrally located δ-opiate receptors and μ-receptors to chronic activation. Postulating (1) a cellular development of tolerance [5], (2) identical binding sites for, e.g., δ-receptors, and (3) the presence of similar receptor-coupled biochemical systems for peripheral and central receptors, then the response of receptors to chronic activation should be similar irrespective of their location within or outside the CNS. However, δ-receptors in the CNS develop a different degree of tolerance to those in the vas deferens. One difference has been already suggested: chronic exposure of the δ-receptors in the vas deferens to DADL may cause an adaptation at the receptor level [20], a mechanism not established as occurrent in the CNS [9]. Furthermore, the development of tolerance to opioids in the mouse vas deferens seems not to be associated with dependence [20], which contrasts with the effect of chronic opiate exposure upon the CNS. Whether or not these differences relate to the localization of the opiate receptors, which are confined to presynaptic sites in the vas deferens, but are very probably at both pre- and postsynaptic sites in the CNS, remains to be elucidated. Anyway, the selective activation of μ- and δ-receptors appears to be much more difficult to attain in the CNS as compared to the vas deferens of the mouse. These considerations, however, hardly explain the observed failure of SUF to selectively activate analgesia-medating μ-receptors, as is revealed by contrasting the results obtained with SUF to those from chronic infusion of DADL.

Apparently, the data do not favour the notion advanced by others that μ-receptors mediate analgesia [3, 18, 21, 23] and δ-receptor certain other behavioural effects [4,22]. It is concluded that analgesia and catatonia may be mediated by both μ- and δ-receptors. In any case, the selective development of tolerance, especially w.r.t. catatonia, underlines the independence of the observed pharmacological effects mediated by different receptor types. This finding is in line with a report of Fields *et al.* [16] that both μ- and δ-receptors appear to modulate the activity of primary afferent fibres in the dorsal horn of the spinal cord. In addition, our data conflict with the interpretation of Pasternak *et al.* [16,17] that μ-receptors do not mediate analgesia.

ACKNOWLEDGEMENTS

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