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Receptor Binding, Analgesic and Antitussive Potency of Tramadol and Other Selected Opioids

By H.-H. Hennies, E. Friderichs, and J. Schneider

Dedicated to Professor Dr. Hubert Giertz on the occasion of his 65th birthday

Summary: *The influence of replacing the phenolic hydroxyl by the methoxy group on opioid receptor binding, analgesic and antitussive action was investigated in the corresponding couples morphine-codeine, hydromorphone-hydrocodone and O-desmethyltramadol (L 235)-tramadol. Binding was studied on rat whole brain membranes (without cerebellum) with the radioligands dihydromorphine (μ-site), ethylketocyclazocine (k-site), D-Ala²-D-Leu⁵-enkephalin (δ-site) and naloxone (no selective binding). Analgesia (tail flick) and antitussive action (NH_3-vapour induced cough) was investigated in rats and ED_{50} values 10 min after i.v. application were calculated to compare efficacy. All free hydroxyl compounds had higher opioid receptor affinities than the corresponding methoxy derivatives and were more active at the μ-site. The methoxy derivatives codeine and tramadol only had low affinities lacking selectivity towards μ-, k-, or δ-binding. Hydrocodone in contrast showed strong and μ-selective binding. The hydroxy compounds had higher analgesic activity than the methoxy congeners and analgesia appeared to correlate with μ-binding affinity. Codeine and hydrocodone were weaker antitussives than the corresponding hydroxy compounds, whereas no significant difference was found between O-desmethyltramadol and tramadol. Only in the tramadol group the methoxy substitution increased antitussive potency in relation to analgesic potency.*

Zusammenfassung: *Rezeptorbindung, analgetische und antitussive Wirksamkeit von Tramadol und anderen ausgewählten Opioiden*

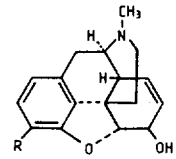
Anhand der Substanzpaare Morphin-Codein, Hydromorphon-Hydrocodon und O-Desmethyltramadol (L 235)-Tramadol wurde untersucht, auf welche Weise der Austausch der phenolischen Hydroxyl-Gruppe gegen eine Methoxy-Gruppe die Opioid-Rezeptorbindung, Analgesie und antitussive Wirksamkeit beeinflußt. Die Opioid-Rezeptorbindungsstudien wurden an Rattenhirnmembranen (Ganzhirn ohne Cerebellum) durchgeführt. Zur Markierung des μ-Rezeptors wurde der Radioligand Dihydromorphin, für den k-Rezeptor Ethylketocyclazocin, und für den δ-Rezeptor D-Ala²-D-Leu⁵-enkephalin verwendet. Als nicht selektiver Radioligand wurde Naloxon eingesetzt, um summarisch alle vorhandenen Bindungsstellen erfassen zu können. Die pharmakologischen Wirkungen Analgesie (tail flick) und antitussive Wirksamkeit (NH_3-induzierter Husten) wurden ebenfalls an der Ratte untersucht und die ED_{50}-Werte jeweils 10 min nach i.v. Applikation der Opioide ermittelt. Generell hatten die Substanzen mit den freien Hydroxy-Gruppen eine höhere Rezeptoraffinität im Vergleich zu den korrespondierenden Methoxy-Derivaten. In allen Fällen wurde eine höhere Selektivität zugunsten der μ-Bindungsstellen beobachtet. Die Methoxy-Derivate Codein und Tramadol hatten nur schwache Affinitäten zu Opioid-Rezeptoren und zeigten keine Selektivität gegenüber μ-, k- oder δ-Bindungsstellen. Im Gegensatz dazu wies Hydrocodon eine ausgesprochen hohe und selektive μ-Bindung auf. Alle Hydroxy-Verbindungen zeigten eine höhere analgetische Aktivität im Vergleich zu den entsprechenden Methoxyverbindungen, wobei die Analgesie mit der Affinität zu den μ-Bindungsstellen korrelierte. Codein und Hydrocodon zeigten eine schwächere antitussive Wirkung als die korrespondierenden Hydroxy-Derivate, während kein signifikanter Unterschied bei dem Substanzpaar O-Desmethyltramadol-Tramadol auftrat. Nur in der Tramadol-Gruppe erhöhte die Methoxy-Substitution die antitussive Potenz im Vergleich zur analgetischen Wirkamkeit.

Key words: *Codeine · O-Desmethyltramadol · Hydrocodone · Hydromorphone · L 235 · Morphine · Tramadol*

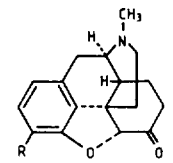
1. Introduction

Despite the ample variability in the structure of opioids, most compounds that behave as narcotic analgesics contain an aromatic ring system spaced from a basic nitrogen center by a group of 2 or 3 carbon atoms (see Beckett and Casy 1954; Casy and Parfitt 1986). To increase efficacy, the aromatic ring can be substituted with an oxygen containing residue, preferably in meta position in respect to the carbon chain. The oxygen containing group is mostly the free phenolic hydroxyl, but lower alkyl ether like e.g. the methoxy group retain appreciable analgesic efficacy (Eddy and May 1973).

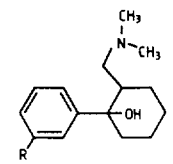
Inhibition of the cough reflex is a useful additional property of opioids and preferentially the methoxy compounds (Eddy and May 1973; Eddy et al. 1969) like codeine, dihydrocodeine, hydrocodone and thebacone are widely accepted as antitussives. Within a group of selected opioids, it was of interest to compare how the replacement of the phenolic hydroxyl by the methoxy group influences the ratio of antitussive to analgesic potency and how the opioid receptor binding and the binding selectivity towards the different species of opioid receptors are changed. For this study the couples morphine-codeine, hydromorphone-hydrocodone and O-desmethyltramadol and tramadol were chosen (see structural formulae). Tramadol is a centrally acting analgesic (Friderichs et al. 1978) which shows some structural similarities to codeine, and the hydroxy analog, O-desmethyltramadol, is one of the tramadol metabolites (Lintz et al. 1981). To avoid species-dependent differences in activity, all investigations (inhibition of cough reflex, antinociceptive action, opioid receptor binding) were performed in the same species, i.e. the rat.



R = OH Morphine
R = OCH_3 Codeine



R = OH Hydromorphone
R = OCH_3 Hydrocodone



R = OH O-Desmethyl-tramadol
R = OCH_3 Tramadol

2. Methods

2.1. Opioid receptor studies in vitro

Male Wistar SPF rats (breeder: Lippsche Versuchstierzucht, Hagemann GmbH, Extertal-Bösingfeld, FR Germany) of ca. 200 g body weight were used. Rat brain membrane suspensions (whole brain less the cerebellum) were prepared essentially as described by Wood et al. (1981). The radioreceptor assays were performed according to the same authors with some minor modifications. The total assay volume of 500 µl contained 0.8—1 mg membrane suspension (protein determination according to Lowry et al. (1951)) and the radioactive ligand. Incubations were initiated by the addition of the tritiated ligand and were carried to equilibrium, namely

at 25°C for 60 min with (^{3}H)-dihydromorphine ((^{3}H)DHM),
at 25 °C for 60 min with (^{3}H)-(D-Ala^2-D-Leu^5)enkephalin ((^{3}H)DADLE),
at 4 °C for 30 min with (±) (^{3}H)ethylketocyclazocine ((^{3}H)EKC),
at 25 °C for 30 min with (^{3}H)-naloxone ((^{3}H)NAL).

The concentrations for the ligands in the assays and the specific radioactivities used were:

[^{3}H]-DHM: 2 nmol/l; 76 Ci/mmol (Amersham, Braunschweig, FR Germany),
[^{3}H]-EKC: 4 nmol/l; 16.4 Ci/mmol (NEN, Dreieich, FR Germany),
[^{3}H]-DADLE: 2 nmol/l; 25 Ci/mmol (Amersham),
[^{3}H]-NAL: 1 nmol/l; 55 Ci/mmol (Amersham).

Blanks to quantify nonspecific binding were obtained by saturating the binding sites in case of:

[^{3}H]-DHM binding with 10^{-6} mol/l morphine
[^{3}H]-EKC binding with 10^{-5} mol/l (—)5,9-dimethyl-2-hydroxy-2-tetrahydrofurfuryl-6,7-benzo-morphan hydrochloride (MR-2184; Boehringer Ingelheim/Rhein, FR Germany),
[^{3}H]-DADLE binding with 10^{-5} mol/l DADLE (Serva, Heidelberg, FR Germany),
[^{3}H]-NAL binding with 10^{-5} mol/l NAL · HCl (ENDO, Garden City, NY, USA).

The incubations were terminated by rapid filtration under mild vacuum and 2 washes with cold buffer using Whatman glass microfibre filters (GF/C, Ø 2,5 cm, Whatman, Maidstone, England).

The radioactivity of the samples was counted after a stabilizatio and extraction period of at least 15 h. In case of [^{3}H]-DADLE binc ing studies, 25 µg bacitracin (Serva) per assay were added. 3—5 cor centrations per test compound in 3—13 independent determinatior (n) per opioid concentration were assayed. Data are reported as IC values and 95 % confidence limits are given in parentheses.

2.2. Tail flick analgesia

Tail flick analgesia (antinociceptive action) was measured again radiant heat, according to D'Amour and Smith (1941), in 80—120 female Sprague-Dawley rats (breeder: Hagemann) with a cut-o time of 12 s. Increase in tail flick latency to ≥ 150 % of the prea plication value was considered to indicate analgesia and ED_{50} valu 10 min after i.v. drug application were calculated according to Litcl field and Wilcoxon (1949).

2.3. Antitussive effect

Wistar SPF rats (breeder: Hagemann) with a body weight of 160- 300 g were used. The animals were placed on the perforated floor a 12 l glass vessel and 1 ml of 8 % aqueous ammonia solution wa poured on to the bottom. The lid of the vessel was fit with a micr phone and the number of coughs was counted for 8 min by mear of a head phone. The test compounds were given intravenously 1 min before ammonia exposition. The regression lines and ED_{50} va ues for the percental decrease in cough rate vs vehicle-treated contr rats were calculated.

2.4. Drugs investigated

The following drugs (commercial source) were used: mo phine · HCl; codeine phosphate; hydromorphone · HCl; hydroc done hydrogentartrate; tramadol · HCl (Grünenthal); O-desmethy tramadol · HCl (L 235; Grünenthal). For animal studies, all drug (doses refer to the appropriate salts) were dissolved in 0.9 % salir and applicated intravenously in a volume of 10 ml/kg body weigh

3. Results and discussion

Table 1 summarizes the IC_{50} values of the different con pounds in displacing the specific binding of radioactiv opioid ligands in rat brain membranes in vitro. In the binc ing assay, we focussed our interest on the functionally mo important receptor types, the µ-, k-, and δ-binding sites. I addition displacement of naloxone as a ligand with affinit to all types of opioid receptors was investigated.

The highest affinity in respect to total opioid binding wit an IC_{50} value of 2.2×10^{-8} mol/l was seen with morphin followed by hydromorphone and hydrocodone, which wer 2 and 4-fold less active, respectively. O-desmethyltramadc had a total opioid binding affinity more than one order c magnitude lower than morphine. Tramadol and codein were even less potent. Their IC_{50} values decreased to th micromolar range and both compounds had more than orders of magnitude lower affinity than morphine. Th methoxy compounds as compared with the correspondir hydroxy congeners showed uniformely a lower total opioi receptor affinity. This difference was very pronounced in th group morphine-codeine, less prominent in the group O-de methyltramadol and tramadol and only minimal in th group hydromorphone-hydrocodone.

Table 1: Potency (IC_{50}-values, mol/l) of different opioids in displacing the specific binding of radioactive ligands in rat brain membranes in vitro (95 % confiden limits in parentheses).

Compound	Ligand			
	[^{3}H]-DHM	[^{3}H]-EKC	[^{3}H]-DADLE	[^{3}H]-NAL
O-Desmethyltramadol	4.4×10^{-7} ($2.3—7.2\times10^{-7}$)	3.9×10^{-6} ($2.6—6.3\times10^{-6}$)	1.5×10^{-6} ($9.8—23.0\times10^{-7}$)	8.8×10^{-7} ($6.1—12.0\times10^{-7}$)
Tramadol	1.7×10^{-6} ($7.4—36.0\times10^{-7}$)	6.5×10^{-6} ($4.0—11.0\times10^{-6}$)	2.2×10^{-6} ($1.1—3.8\times10^{-6}$)	6.1×10^{-6} ($4.0—9.5\times10^{-6}$)
Morphine	4.6×10^{-9} ($3.0—7.9\times10^{-9}$)	5.6×10^{-7} ($2.3—10.0\times10^{-7}$)	1.1×10^{-8} ($6.8—17.0\times10^{-9}$)	2.2×10^{-8} ($1.1—3.6\times10^{-8}$)
Codeine	1.5×10^{-6} ($8.6—30.0\times10^{-7}$)	2.7×10^{-6} ($1.4—5.3\times10^{-6}$)	5.8×10^{-6} ($3.0—12.0\times10^{-6}$)	3.7×10^{-6} ($2.3—5.6\times10^{-6}$)
Hydromorphone	7.7×10^{-9} ($3.4—15.0\times10^{-9}$)	1.1×10^{-8} ($3.4—27.0\times10^{-9}$)	6.1×10^{-7} ($2.7—12.0\times10^{-7}$)	4.6×10^{-8} ($2.4—7.4\times10^{-8}$)
Hydrocodone	5.5×10^{-8} ($2.1—11.0\times10^{-8}$)	4.1×10^{-7} ($2.6—6.3\times10^{-7}$)	1.8×10^{-7} ($9.2—32.0\times10^{-8}$)	8.7×10^{-8} ($2.8—18.5\times10^{-8}$)

Regarding the selectivity towards the different types of opioid receptors, it was found that the two compounds with the lowest total binding affinities (tramadol and codeine) showed this low affinity at all receptor types investigated. The other compounds exhibited a more or less pronounced selectivity for the μ-type opioid receptor. The affinity of morphine decreased in the order μ-, δ- and k-receptor, δ-binding was 2.4-fold weaker and k-binding 122-fold weaker than μ-binding. Hydromorphone binding decreased in the order μ-, k-, δ-receptor. The difference of μ- to k-binding was only very small (1.4-fold decrease) but pronounced in respect to δ-binding (79-fold decrease). O-desmethyltramadol and hydrocodone showed no difference between k- and δ-affinities which in both cases were about 10-fold lower than the corresponding μ-affinity.

Analgesic efficacy was investigated in the tail flick test (Fig. 1). All compounds dose-dependently increased the number of animals with analgesia and the slopes of the dose-response curves were nearly identical. As shown by the ED_{50} values (Table 2), the antinociceptive activity decreased in the order hydromorphone > hydrocodone > morphine > O-desmethyltramadol > codeine = tramadol. Within the corresponding couples, the methoxy compound was uniformly less active than the hydroxy congener.

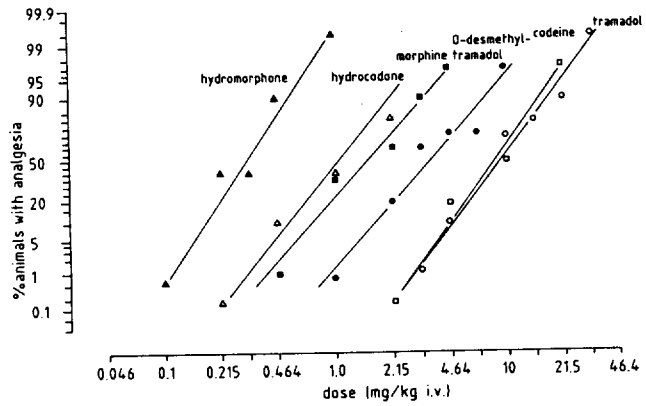


Fig. 1: Dose-response curve of analgesia (antinociceptive action) in the rat tail flick test. Analgesia (increase in tail flick latency to > 150 % of pretreatment value) was rated 10 min after i.v. drug application, n = 10/dose.

Table 2: ED_{50}-values (mg/kg) of analgesic and antitussive effects in conscious rats 10 min after i.v. application (confidence limits in parantheses). Ratio of antitussive to analgesic potency was calculated by dividing ED_{50}-value of tail flick by ED_{50}-value of cough inhibition.

Compound	Analgesic effect	Antitussive effect	Ratio
O-Desmethyltramadol	2.94 (2.03—4.26)	4.31 (30.5—6.87)	0.68
Tramadol	8.97 (6.20—13.0)	3.52 (2.68—4.74)	2.55
Morphine	1.37 (0.80—2.34)	3.89 (2.72—6.03)	0.35
Codeine	8.60 (5.76—12.8)	13.0 (8.66—28.0)	0.66
Hydromorphone	0.28 (0.20—0.38)	0.29 (0.23—0.35)	0.97
Hydrocodone	1.18 (0.75—1.85)	1.87 (1.47—2.30)	0.66

As tail flick analgesia is regarded to be mostly effected by interaction with the μ-type of opioid receptor (Zukin and Zukin 1981; Herz 1984), it was investigated how far μ-binding affinity would correspond with analgesic efficacy. Fig. 2 shows a correlation analysis between IC_{50} values of μ-binding and ED_{50} values in the tail flick test. A correlation coefficient of 0.883 was calculated which indicates a fairly good correlation between the two parameters.

However, in quantitative terms considerable discrepancies between μ-receptor affinity and analgesic potency remains obvious. In this context it should not be overlooked that the binding studies, due to only partial selectivity of the labeled ligands (Wood et al. 1981; Goldstein and James 1984), may still represent hybrid affinities to multiple receptor types. Also analgesia, measured as antinociception in the tail flick

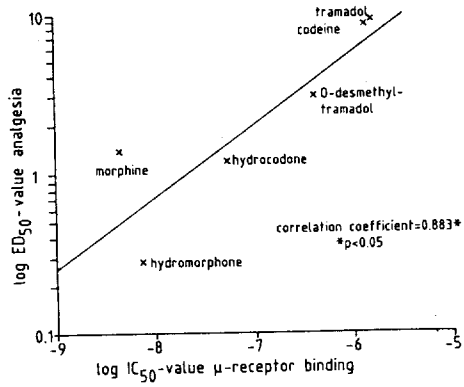


Fig. 2: Correlation analysis of analgesic potency with μ-receptor binding. ED_{50} values of tail flick test and IC_{50} values of displacement of [^{3}H]-dihydromorphine binding, both in rats, were compared.

test, may depend on interference with more than one receptor type (Vaught et al. 1982) and includes extracerebral targets like the spinal cord (Yeung and Rudy 1980). In addition, non-opioid-like actions may be involved. Recently, e.g. a naloxone insensitive antinociceptive effect of tramadol on ascending spinal cord activities was described (Carlsson and Jurna, submitted). Finally, in contrast to the binding studies in brain homogenate, analgesic efficacy is strongly influenced by the pharmacokinetic properties of the individual compounds.

Antitussive efficacy was investigated against ammonia-induced cough reaction (Fig. 3). A dose-dependent cough inhibition was induced by all compounds and corresponding to antinociception, the slopes of the antitussive dose response curves did not substantially differ from each other. Efficacy decreased in the order hydromorphone > hydrocodone > tramadol = morphine = O-desmethyltramadol > codeine (Table 2). Methoxy as compared to hydroxy substitution did not increase the antitussive efficacy.

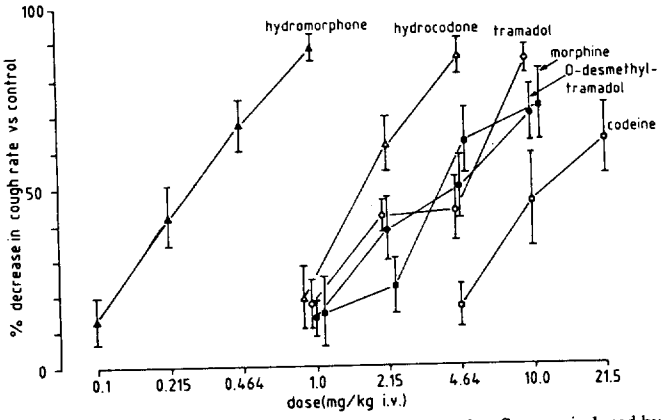


Fig. 3: Dose-response curve of antitussive action. Cough reflex was induced by ammonia vapour in conscious rats 10 min after intravenous drug application, $\bar{x} \pm sx$, n = 8/dose.

By using the correlation analysis we checked if antitussive action corresponds with affinity at one of the opioid receptor types investigated. The correlation analysis revealed no correlation with δ-binding (corr. coeff. = 0.265) a weak correlation with μ-binding (corr. coeff. = 0.638) and astonishingly a high correlation with k-binding (corr. coeff. = 0.872; data not shown). This might suggest that k-binding and perhaps μ-binding may implicate antitussive action, although this finding is based on a very limited number of compounds. A possible link between k-binding and antitussive action was already mentioned by Chau et al. (1983). As a model for antitussive opioid receptors, they measured saturable binding of codeine in crude guinea pig medulla homogenate (Chau et al. 1982). They observed a good correlation between codeine displacement in vitro and antitussive action in vivo and k-agonists like ketazocine and cyclazocine were more potent than μ-opioids in displacing codeine from the

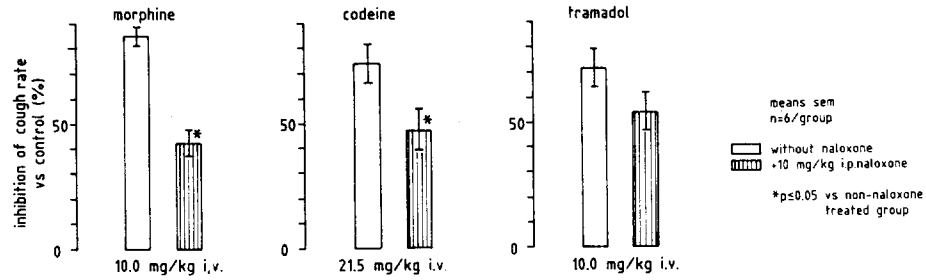


Fig. 4: Influence of pretreatment with 10 mg/kg i.p. naloxone on the antitussive action of morphine, codeine and tramadol in rats.

binding sites in the medulla homogenate. But extensive investigations with the optical isomers of opioids (Winter and Flataker 1952; Pellmont and Bächtold 1954; Robbins and Miller 1960; Chau and Harris 1980) and interaction studies with naloxone (Chau et al. 1983; Kamei et al. 1986) and other opioid antagonists (Kasé et al. 1976) indicate, that receptors mediating cough inhibition are clearly different from analgesic opioid receptors like the μ- or k-type. In general, structural requirements for antitussive efficacy are less rigorous than those for analgesia. Also less stereoselectivity (Chau et al. 1982, 1983) is usually required for antitussive than for analgesic effects (Beckett and Casy 1954; Casy and Parfitt 1986). Naloxone unequivocally antagonizes the analgesic action of opioids whereas cough inhibition, depending on the experimental model or the individual compound investigated, is more or less insensitive to naloxone (Chau et al. 1983, Cavanagh et al. 1976). This is confirmed by our own results (Fig. 4). Only the effect of morphine in ammonia-induced cough is fairly well antagonized by a high dose of naloxone. Cough inhibition by codeine is only moderately and that by tramadol not significantly inhibited by the naloxone pretreatment.

As opioids with a methoxy group like e.g. codeine, thebacone, hydrocodone are preferentially used as antitussives (Eddy and May 1973), these opioids were investigated for selectivity of antitussive action. In Table 2 the ratio of antitussive to analgesic potency is depicted. The methoxy compound tramadol reaches a value of 2.55 indicating 2- to 3-fold stronger antitussive than analgesic potency, whereas the hydroxy compound morphine with a value of 0.35 has a substantially stronger analgesic than antitussive potency. All other compounds showed statistically indistinguishable ED_{50} values in both parameters. Therefore no general prevalence for antitussive action can be attributed to compounds with the methoxy group.

A rational why codeine and hydrocodone are preferentially used as antitussives and morphine and hydromorphone as analgesics is thus not obvious. Possibly the global decrease in opioid receptor affinity of the methoxy compounds, implying less severe opioid side effects and less dependence potential, makes them safer antitussives than the corresponding hydroxy compounds.

4. References

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