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EFFECTS OF STRUCTURAL MODIFICATIONS OF N-CPM-NORMORPHINE DERIVATIVES ON AGONIST AND ANTAGONIST ACTIVITIES IN ISOLATED ORGANS*

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The agonistic and antagonistic properties of N-cyclopropylmethyl (N-CPM) morphine derivatives were observed in mouse vas deferens (MVD), longitudinal muscle of guinea pig ileum (GPI) and rabbit vas deferens (LVD). In MVD the K_e values of the titled compounds (N-CPM-morphine, N-CPM-isomorphine, N-CPM-dihydromorphine, N-CPM-dihydroisomorpPhine, N-CPM-dihydromorphone and naltrexone) were measured for μ-, κ- and δ-receptors using normorphine, ethylketocyclazocine (EKC) and D-Pen^2-D-Pen^5-enkephaline (DPDPE) as selective agonists on the receptors, respectively. For μ-receptors of MVD the tested compounds showed similar affinity. For κ-receptors the non-iso-6-OH derivatives possessed much less affinity than the iso-derivatives. Similar difference could be observed for δ-receptors. The agonistic activities of these compounds in MVD were observed to be between 0–20% of the inhibition of muscle contractions. In GPI the compounds – except naltrexone – possessed strong agonistic activities effectively antagonized by nor-binaltorphimine (nor-BNI) (K_e of nor-BNI was 0.23 nM) suggesting that they were strong κ-receptor agonists. We investigated these agents in LVD too, which contains κ-receptors, but they did not produce any agonist potencies. It raises the possibility that the κ-receptor subtypes of LVD and MVD are different from the κ-receptor subtype of GPI or the vasa deferentia contain much fewer κ-receptors than GPI and the intrinsic activities of these compounds are too small to reach the 50% inhibition of the contractions.

Keywords: N-CPM-morphines – opioid receptors – isolated organs

INTRODUCTION

N-cyclopropylmethylnorazidoisomorphine (CAM) produced both agonist and antagonist properties in vitro and in vivo. Based on its potent agonist activity in the rabbit vas deferens and the low potency of naloxone to antagonize its effects in the guinea pig ileum and on its diuretic effect in the rat CAM was suggested to be κ-receptor agonist. Other data showed that CAM exhibits antagonist activities on μ and δ opioid receptors [2]. The aims of our present experiments were to determine:

1. How the replacement of the methyl group by cyclopropylmethyl (CPM) group on the nitrogen atom of morphine (N-CPM-morphine, **[a]**) changes the activity profile?

* Dedicated to Professor Maria Wollemann on the occasion of her 80th birthday.

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2. How the saturation of double bond at $C_{7–8}$ position (N-CPM-dihydromorphine, **[c]**) and the epimerization of hydroxyl groups at C_6 position (N-CPM-isomorphine, **[b]**, N-CPM-dihydroisomorphine, **[d]**) influence the in vitro activities?

3. How does the OH-group in the position C_{14} change the agonist and antagonist activities of N-CPM-dihydromorphone, **[e]**)?

4. What type of opioid receptors are involved in the pharmacological effects of the N-CPM-morphine derivatives?

MATERIALS AND METHODS

Isolated guinea pig ileum (GPI)

The longitudinal muscle with myenteric plexus of guinea pig ileum was prepared and incubated in Krebs-solution in 37 °C [5]. The inhibitory action of opioid agonists on the electrically (0.1 Hz, 1 ms supramaximal stimulus) evoked contractions were measured and IC_{50} values were estimated for the agonist activity and K_e values for the antagonist activity in the presence of 1–2 nM norbinaltorphimine (nor-BNI).

Isolated mouse vas deferens (MVD)

The mouse vas deferens was prepared from CFLP male mice and incubated in Mg^{++}-free Krebs-solution in 31 °C [3]. The inhibitory action of opioid agonists on the electrically evoked contractions (10 Hz, 2 impulses, supramaximal stimulus in every 10 s) were measured and IC_{50} values were estimated for the agonist activity and K_e values for the antagonist activity.

Isolated rabbit vas deferens (LVD)

The rabbit vas deferens was prepared from New-Zealand White rabbits and incubated in Mg^{++}-free Krebs-solution in 35 °C [4]. The inhibitory action of opioid agonists on the electrically evoked contractions (0.1 Hz 1 ms supramaximal stimulus) were measured and IC_{50} values were estimated for the agonist activity.

RESULTS AND DISCUSSION

Substitution of N-methyl group by CPM of the tested compounds greatly increased the agonist potencies in the GPI preparation. The rank order of agonist activities were: N-CPM-isomorphine > N-CPM-morphine > N-CPM-dihydromorphine > N-CPM-dihydroisomorphine (Table 1). The antagonist potencies of naloxone against N-CPM compounds (K_e: 6.2–7.1 nM) were found to be lower than those of against

Table 1
The agonist potencies of the N-cyclopropylmethyl (N-CPM) derivatives in the isolated guinea pig ileum preparation

Compound	IC_{50} nM ± S.E.M.	Relative agonist potency (morphine = 1)
a	1.8 ± 0.30	40
b	1.3 ± 0.26	52
c	2.4 ± 0.16	28
d	2.9 ± 0.67	23

Table 2
The antagonist potencies of naloxone and nor-BNI against N-cyclopropylmethyl (N-CPM) derivatives in the isolated guinea pig ileum preparation

Compound	K_e of naloxone (nM) ± S.E.M.	K_e of nor-BNI (nM) ± S.E.M.
a	7.1 ± 0.9	0.25 ± 0.11
b	6.2 ± 0.7	0.22 ± 0.04
c	6.6 ± 1.2	0.23 ± 0.08
d	6.8 ± 1.7	0.15 ± 0.06

n = 3 – 4
K_e of nor-BNI against normorphine: 12.6 nM

N-methyl derivatives (K_e: 1.1–1.2 nM) and nor-BNI antagonized the agonist activity of N-CPM compounds (K_e: 0.15–0.25 nM) more effectively (Table 2) than that of morphine (K_e: 12.6 nM) in the GPI preparation. The low antagonist activity of naloxone and the potent antagonist activity of nor-BNI against the agonist action of the N-CPM compounds suggest that their agonist effects might be mediated through κ-receptors. While the replacement of the N-methyl moiety by CPM group greatly increased the affinity of the investigated compounds for μ-receptors, abolished their μ-agonist activity, which is characteristic for N-methylated parent molecules in the GPI and MVD preparations [1]. In the GPI the C_{14}-hydroxyl derivative of N-CPM-dihydromorphone (naltrexone) did not show any agonist activity in two times higher concentration than the IC_{50} of the parent compound. In the MVD preparation the concentration-effect curves of N-CPM compounds were found to be shallow and failed to reach the 50% inhibition (data not shown).

In the GPI preparation all of the N-CPM compounds were found to be potent antagonists against normorphine in the presence of nor-BNI a highly selective κ-receptor antagonist (Table 3). Their antagonist potencies against normorphine were similar. In the MVD preparation the N-CPM compounds showed antagonist activity against the μ-receptor agonist normorphine, the κ-receptor agonist ethylketocyclazocine (EKC) and the δ-receptor agonist (D-Pen^2-D-Pen^5)-enkephalin (DPDPE). The N-CPM compounds showed the highest affinity for the μ-receptors and the lowest

Table 3
The antagonist potencies of the N-CPM derivatives in the isolated guinea pig ileum preparation

Compound	K_e(nM)* ± S.E.M. against normorphine	Relative antagonist potency (naloxone = 1)
a*	1.10 ± 0.12	1.10
b*	1.50 ± 0.39	0.80
c*	0.85 ± 0.15	1.40
d*	0.81 ± 0.15	1.50
e*	1.58 ± 0.30	0.76
naltrexone	1.56 ± 0.10	0.78

*K_e estimated in the presence of 1–2 nM nor-BNI
n = 3 – 4

affinity for δ-receptors. N-CPM-morphine and N-CPM-dihydromorphine showed relatively selective antagonist activity on μ-receptors; their affinities for μ-receptors were 33 and 21 times higher than for κ-receptors, respectively, and 38 and 40 times higher than for δ-receptors, respectively. The epimerization of the C_6-OH does not change significantly the antagonist potencies of N-CPM-morphine and N-CPM-dihydromorphine on μ-receptors in the GPI and MVD preparation, but it increased the affinities of these compounds for κ- and δ-receptors. N-CPM-isomorphine and N-CPM-dihydroisomorphine proved to be less selective antagonists on μ-receptors than the parent compounds. The saturation of $C_{7–8}$ double bond of N-CPM-morphine and N-CPM-isomorphine caused minimal changes in the agonist and antagonist potencies. The affinity of N-CPM-dihydromorphone for κ-receptors was similar to that of N-CPM-dihydroisomorphine but that for δ-receptors was higher than the affinity of the other N-CPM compounds (Table 4). It can be concluded that the modification of C_6-OH group to C_6-oxo group in the N-CPM-morphine derivatives decreases the receptor selectivity between κ- and δ-receptors. In LVD the N-CPM C_6-OH and C_6-oxo compounds did not show any agonist activity in contrast to CAM

Table 4
Affinities of N-CPM-morphine derivatives for the opioid receptors of mouse vas deferens (MVD).
K_e(nM) ± S.E.M.

Compound	μ (NM)	κ (EKC)	δ (DPDPE)
a	1.87 ± 0.42	52.87 ± 7.48	89.1 ± 3.20
b	1.47 ± 0.28	5.57 ± 0.76	22.3 ± 1.58
c	1.57 ± 0.14	35.90 ± 3.78	70.8 ± 5.12
d	0.89 ± 0.20	7.34 ± 0.64	26.5 ± 5.74
e	0.46 ± 0.05	7.46 ± 1.54	7.8 ± 0.98
naltrexone	0.79 ± 0.04	N.D.	14.9 ± 0.58

NM = normorphine, N.D. = not determined
n = 4 – 6

and bremazocine (data not shown). The partial agonist effect in the MVD and the lack of the agonist effect in the LVD of N-CPM compounds in contrast to the full agonist action of EKC could be explained either by their low efficacy on κ-receptors or the existence of different subtypes of κ-receptors in the MVD, LVD and GPI.

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