

Inhibition of striatal and retinal dopamine release *via* nociceptin/orphanin FQ receptors

¹K. Flau, ¹A. Redmer, ¹S. Liedtke, ¹M. Kathmann & ^{*,1}E. Schlicker

¹Institut für Pharmakologie und Toxikologie, Universität Bonn, Reuterstr. 2b, 53113 Bonn, Germany

1 We determined the effects of nociceptin/orphanin FQ and the NOP receptor ligands acetyl-Arg-Tyr-Tyr-Arg-Ile-Lys-NH₂ (Ac-RYYRIK-NH₂) and naloxone benzoylhydrazone on transmitter release *in vitro*.

2 The electrically evoked tritium overflow from guinea-pig and mouse striatal slices and guinea-pig retinal discs preincubated with [³H]-dopamine was inhibited by nociceptin/orphanin FQ (pEC₅₀ 7.9, 7.6 and 8.6; E_{max} 30, 50 and 55%). Ac-RYYRIK-NH₂ 0.032 μM and naloxone benzoylhydrazone 5 μM antagonized the effect of nociceptin/orphanin FQ in striatal slices of the guinea-pig (apparent pA₂ 9.1 and 6.8) and the mouse (apparent pA₂ 9.2 and 7.5) and strongly attenuated the effect of nociceptin/orphanin FQ 0.1 μM in guinea-pig retinal discs. Ac-RYYRIK-NH₂ 0.032 μM did not affect the evoked overflow by itself whereas naloxone benzoylhydrazone 5 μM inhibited it in each tissue.

3 The electrically evoked tritium overflow from mouse brain cortex slices preincubated with [³H]-noradrenaline was inhibited by nociceptin/orphanin FQ (pEC₅₀ 7.9, E_{max} 85%), Ac-RYYRIK-NH₂ (pEC₅₀ 8.3, E_{max} 47%) but not affected by naloxone benzoylhydrazone 5 μM. Ac-RYYRIK-NH₂ and naloxone benzoylhydrazone showed apparent pA₂ values of 8.6 and 6.9.

4 In conclusion, the inhibitory effect of nociceptin/orphanin FQ on dopamine release in the striatum and retina and on noradrenaline release in the cerebral cortex is mediated *via* NOP receptors. Ac-RYYRIK-NH₂ behaves as an extremely potent NOP receptor antagonist in the striatum and retina and as a partial agonist in the cortex.

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Keywords: NOP receptor; nociceptin; orphanin FQ; dopamine release; noradrenaline release; striatum; guinea-pig retina; mouse cerebral cortex; Ac-RYYRIK-NH₂; naloxone benzoylhydrazone

Abbreviations: Ac-RYYRIK-NH₂, acetyl-Arg-Tyr-Tyr-Arg-Ile-Lys-NH₂; [³H]-DA, [³H]-dopamine; [³H]-NA, [³H]-noradrenaline; NalBzOH, naloxone benzoylhydrazone; N/OFQ, nociceptin/orphanin FQ; PSS, physiological salt solution

Introduction

A fourth type of opioid receptor, which has a homology in the amino acid sequence to the three classical opioid receptors of about 50%, was first identified in 1994 (for review, see Mogil & Pasternak, 2001). This receptor, termed NOP according to the nomenclature of IUPHAR (International Union of Pharmacology; Cox *et al.*, 2000), is activated by a heptadecapeptide, termed nociceptin/orphanin FQ (N/OFQ) (for review, see Mogil & Pasternak, 2001). The NOP and the classical opioid receptors resemble each other with respect to the transduction mechanisms, i.e. inhibition of adenylyl cyclase (Hawes *et al.*, 2000) and of voltage-dependent Ca²⁺ channels and activation of voltage-dependent K⁺ channels (Moran *et al.*, 2000). However, the NOP receptor markedly differs from the classical opioid receptors in its pharmacological properties (e.g. lack of antagonistic effect of the opioid receptor blocking agent naloxone) (Calo *et al.*, 2000) and its distribution in the brain (Mollereau & Mouldous, 2000). The most striking difference, however, occurs with respect to the influence on nociception. Thus, N/OFQ elicits hyperalgesia rather than analgesia when administered at a supraspinal site (for review, see Mogil &

Pasternak, 2001). The action of N/OFQ is, however, not restricted to nociception since the heptadecapeptide also affects feeding (Polidori *et al.*, 2000), learning and memory (Noda *et al.*, 2000) and anxiety (Jenck *et al.*, 1997) as well as numerous vegetative functions (Mogil & Pasternak, 2001).

One aspect in which the classical opioid and the NOP receptors resemble each other is that they occur presynaptically on a variety of peripheral and central neurones where they cause inhibition of the release of the respective neurotransmitter (Moran *et al.*, 2000; Schlicker & Morari, 2000). N/OFQ e.g. affects the release of dopamine and this has been shown by direct measurement of transmitter release or electrophysiological techniques for the (i) nigrostriatal (Konya *et al.*, 1998; Schlicker *et al.*, 1998a; Shieh & Pan, 2001), (ii) mesolimbic (Murphy *et al.*, 1996; Murphy & Maidment, 1999; Lutfy *et al.*, 2001; Shieh & Pan, 2001; Zheng *et al.*, 2002), (iii) tuberoinfundibular (Wagner *et al.*, 1998; Shieh & Pan, 2001) and (iv) incertohypothalamic system (Shieh & Pan, 2001). The involvement of NOP receptors in the effect of N/OFQ has so far been proven only in the studies by Shieh & Pan (2001; use of an antisense oligodeoxynucleotide) and by Zheng *et al.* (2002; use of the weakly potent NOP receptor antagonist [Phe¹Ψ(CH₂-NH)Gly²]-N/OFQ(1-13)NH₂). The need for such experiments

*Author for correspondence; E-mail: e.schlicker@uni-bonn.de

is highlighted by a study on conscious rats in which intrastriatal administration of N/OFQ increased dopamine release in a manner sensitive to naloxone thereby arguing against the involvement of NOP receptors (Konya *et al.*, 1998).

We therefore examined in two *in vitro* models of the striatum, i.e. superfused guinea-pig and mouse striatal slices, in which N/OFQ inhibits dopamine release in the presence of naloxone (Schlicker *et al.*, 1998a), whether this effect is counteracted by NOP receptor antagonists. We used the selective and highly potent NOP receptor antagonist acetyl-Arg-Tyr-Tyr-Arg-Ile-Lys-NH₂ (Ac-RYYRIK-NH₂) (Dooley *et al.*, 1997; Berger *et al.*, 1999) and a non-selective and less potent NOP receptor antagonist, naloxone benzoylhydrazone, used by our own group (Schlicker *et al.*, 1998b; Malinowska *et al.*, 2000a) and by other investigators (Bucher, 1998; Siniscalchi *et al.*, 1999; Ho *et al.*, 2000; Bigoni *et al.*, 2002). Furthermore, we studied whether N/OFQ affects dopamine release also in guinea-pig retinal discs since the influence of N/OFQ on the retinal dopamine system has so far not been examined. Finally, we compared the potencies of Ac-RYYRIK-NH₂ and naloxone benzoylhydrazone at the NOP receptor causing inhibition of *noradrenaline* release in the mouse brain cortex, i.e. at an NOP receptor previously identified and characterized by our group (Schlicker *et al.*, 1998b; Bauer *et al.*, 1999; Werthwein *et al.*, 1999).

Methods

Superfusion studies

Male Dunkin-Hartley guinea-pigs and male NMRI mice were decapitated and the brains and/or eyes were removed from the skull. The retina was carefully detached from other layers of the guinea-pig eye using a spatula and discs (diameter 3 mm) were punched out. Furthermore, 0.3 mm thick slices were prepared from the guinea-pig and mouse striatum (diameter 2 mm) and the mouse cerebral cortex (diameter 3 mm). Retinal discs and striatal slices were incubated for 30 min with physiological salt solution (PSS) of 37°C containing [³H]-dopamine 100 nM; cortex slices were incubated for 60 min with PSS of 37°C containing [³H]-noradrenaline 25 nM. After incubation, discs or slices were superfused with PSS of 37°C for 110 min at a flow rate of 1 ml min⁻¹. The superfusate was collected in 5 min samples. The PSS was composed as follows (mM): NaCl 118, KCl 4.8, NaHCO₃ 25, KH₂PO₄ 1.2, MgCl₂ 1.2, CaCl₂ 1.3, glucose 11.1, ascorbic acid 0.06, Na₂EDTA 0.03; it was aerated with 95% O₂ and 5% CO₂.

Tritium overflow was evoked by two 2 min periods of electrical field stimulation 40 (S₁) and 90 min (S₂) after onset of superfusion. The stimulation parameters were 1 Hz 200 mA, 2 ms for discs or slices preincubated with [³H]-dopamine and 0.3 Hz, 50 mA, 2 ms for slices preincubated with [³H]-noradrenaline. Usually, N/OFQ or tetrodotoxin was added to (or Ca²⁺ ions were omitted from) the PSS from 62 min of superfusion onward whereas other drugs were present in the PSS throughout superfusion. At the end of superfusion, the radioactivity of the discs and slices and of the superfusate samples was determined by liquid scintillation counting.

Tritium overflow was calculated as the fraction of the tritium content of the slices at the beginning of the respective

collection period (fractional rate of tritium efflux). Basal tritium efflux was quantified by calculating the ratio of the fractional rate in the 5-min period immediately before S₂ (i.e. from 85–90 min; t₂) over that in the collection period from 55–60 min (t₁). Stimulation-evoked tritium overflow was calculated by subtraction of the basal from the total tritium efflux during stimulation and the subsequent 13 min and was expressed as per cent of tritium present in the slice at the onset of stimulation (basal tritium efflux was assumed to decline linearly from the 5 min collection period before to that 15 to 20 min after onset of stimulation). To quantify the effect of N/OFQ on the stimulated tritium overflow, the ratio of the overflow evoked by S₂ over that evoked by S₁ was determined. To determine the effects of antagonists on the evoked overflow, the S₁ values obtained in the presence and absence of the respective antagonist were compared. To quantify agonist potencies, pEC₅₀ values (negative logarithms of the concentration causing the half-maximal effect) were determined. Apparent pA₂ values for antagonists were calculated according to formula 4 of Furchgott (1972).

Statistical analysis

Results are given as means ± s.e.mean of *n* experiments. Student's *t*-test was used for comparison of mean values; if more than one experimental series was compared to the same control, the Bonferroni correction was applied.

Drugs used

[Ring-2,5,6-³H]-dopamine (specific activity (spec. act.) 53.6–60.0 Ci mmol⁻¹), (*R*)-(–)-[ring-2,5,6-³H]-noradrenaline (spec. act. 56.3 Ci mmol⁻¹; NEN, Zaventem, Belgium); acetyl-Arg-Tyr-Tyr-Arg-Ile-Lys-NH₂ (Ac-RYYRIK-NH₂), nociceptin/orphanin FQ (N/OFQ; Bachem, Heidelberg, Germany); desipramine hydrochloride (Ciba-Geigy, Wehr, Germany); naloxone hydrochloride, naloxone benzoylhydrazone, (–)-sulpiride (Sigma, München, Germany); nomifensine (Hoechst, Frankfurt); rauwolscine hydrochloride, tetrodotoxin (Roth, Karlsruhe, Germany). Stock solutions of the drugs were prepared with water, citrate buffer (0.1 mM, pH 4.8; tetrodotoxin) or ethanol (naloxone benzoylhydrazone) and diluted with PSS to the concentration required. The solvents did not affect basal and evoked tritium overflow by themselves.

Results

Superfusion experiments on striatal slices and retinal discs

Guinea-pig and mouse striatal slices and guinea-pig retinal discs were preincubated with [³H]-dopamine and superfused with medium routinely containing nomifensine 10 µM, (–)-sulpiride 3.2 µM and naloxone 10 µM. Basal tritium overflow was not affected or slightly altered (<22%) by addition of tetrodotoxin, N/OFQ, Ac-RYYRIK-NH₂ and naloxone benzoylhydrazone and by omission of Ca²⁺ ions (not shown). Under control conditions (i.e. in the absence of tetrodotoxin, N/OFQ, Ac-RYYRIK-NH₂ and naloxone benzoylhydrazone and in the presence of Ca²⁺ ions), the electrically (1 Hz) evoked tritium overflow (S₂/S₁) amounted to 0.79 ± 0.03 in 20 guinea-pig striatal slices, to 0.76 ± 0.03 in 11 mouse striatal

slices and to 0.87 ± 0.07 in 12 guinea-pig retinal discs. The amount of tritium evoked by S_1 under various experimental conditions is given in Table 1.

Addition of tetrodotoxin $1 \mu\text{M}$ or omission of Ca^{2+} ions before and during S_2 inhibited the evoked tritium overflow (S_2/S_1) by at least 90% in each of the three tissues ($n=4-5$; results not shown). N/OFQ (added to the medium before and during S_2) concentration-dependently inhibited the evoked tritium overflow in each of the three tissues (Figure 1). The maximum inhibitory effect amounted to about 30, 50 and 55% for guinea-pig and mouse striatal slices and guinea-pig retinal discs, respectively; for pEC_{50} values, see Table 2. Ac-RYYRIK-NH₂ $0.032 \mu\text{M}$ (present in the medium throughout superfusion) shifted to the right the concentration-response curves of N/OFQ in guinea-pig (Figure 2A) and mouse striatal slices (Figure 2C), yielding apparent pA_2 values of about 9 (Table 2). The effect of N/OFQ was also antagonized by naloxone benzoylhydrazone $5 \mu\text{M}$ in either tissue (Figure 2B,D); the apparent pA_2 values were about 7 (Table 2). The inhibitory effect of N/OFQ $0.1 \mu\text{M}$ on the electrically evoked tritium overflow in guinea-pig retinal discs was markedly attenuated ($P < 0.015$) by Ac-RYYRIK-NH₂ $0.032 \mu\text{M}$ and abolished by naloxone benzoylhydrazone $5 \mu\text{M}$ (Figure 3). Ac-RYYRIK-NH₂ $0.032 \mu\text{M}$ by itself did not affect the evoked tritium overflow (S_1) whereas naloxone benzoylhydrazone $5 \mu\text{M}$ inhibited it by about 30% in guinea-pig and mouse striatal slices and guinea-pig retinal discs (Table 1).

Superfusion experiments on cerebral cortex slices

Mouse cortex slices were preincubated with [³H]-noradrenaline and superfused with medium routinely containing desipramine $1 \mu\text{M}$, rauwolscine $1 \mu\text{M}$ and naloxone $10 \mu\text{M}$. Basal tritium overflow was not affected by N/OFQ, Ac-RYYRIK-NH₂ and naloxone benzoylhydrazone (not shown). Under control conditions (i.e. in the absence of N/OFQ, Ac-RYYRIK-NH₂ and naloxone benzoylhydrazone), the electrically (0.3 Hz) evoked tritium overflow (S_2/S_1) amounted to 1.15 ± 0.05 in six mouse cortex slices. The amount of tritium evoked by S_1 under various experimental conditions is given in Table 1.

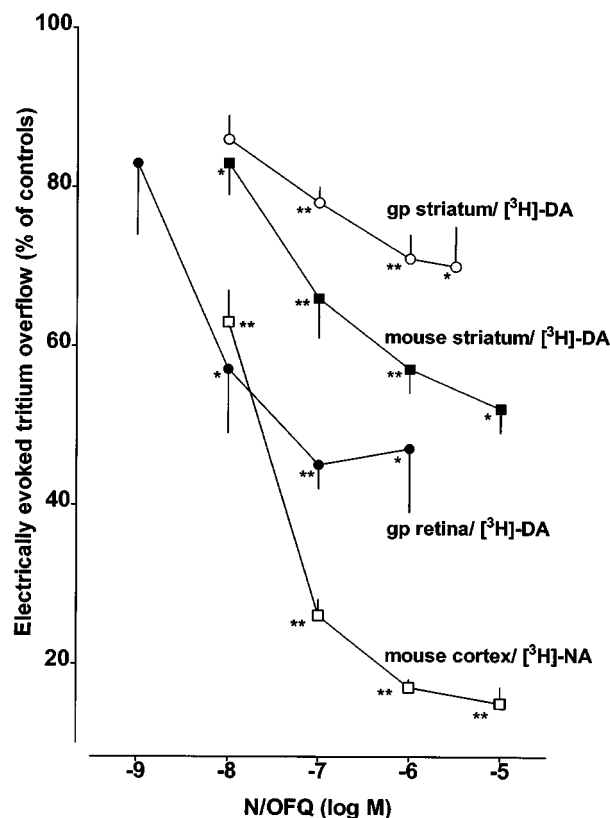


Figure 1 Effect of nociceptin/orphanin FQ (N/OFQ) on the electrically evoked tritium overflow from superfused brain slices and retinal discs. Guinea-pig (gp) and mouse striatal slices and guinea-pig retinal discs preincubated with [³H]-dopamine ([³H]-DA) were superfused with medium containing nomifensine $10 \mu\text{M}$, (–)-sulpiride $3.2 \mu\text{M}$ and naloxone $10 \mu\text{M}$; tritium overflow was evoked at 1 Hz. Mouse cortex slices preincubated with [³H]-noradrenaline ([³H]-NA) were superfused with medium containing desipramine $1 \mu\text{M}$, rauwolscine $1 \mu\text{M}$ and naloxone $10 \mu\text{M}$; tritium overflow was evoked at 0.3 Hz. N/OFQ was added to the medium from 62 min of superfusion onward. Tritium overflow was evoked twice, after 40 (S_1) and 90 min (S_2) of superfusion, and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined. Tritium overflow is given as per cent of the S_2/S_1 value in the corresponding controls (not shown). Means \pm s.e.mean of 5–19 experiments. * $P < 0.05$, ** $P < 0.001$.

Table 1 Effect of Ac-RYYRIK-NH₂ and naloxone benzoylhydrazone on the electrically evoked tritium overflow from superfused brain slices or retinal discs preincubated with [³H]-dopamine or [³H]-noradrenaline

	S_1 (tritium overflow evoked by electrical stimulation; % of tissue tritium)			
	Guinea-pig striatal slices preincubated with [³ H]- dopamine	Mouse striatal slices preincubated with [³ H]- dopamine	Guinea-pig retinal discs preincubated with [³ H]- dopamine	Mouse brain cortex slices preincubated with [³ H]- noradrenaline
Control	5.9 ± 0.3 (25)	8.0 ± 0.5 (12)	4.8 ± 0.4 (25)	8.7 ± 0.6 (5)
Ac-RYYRIK-NH ₂ $0.032 \mu\text{M}$	6.1 ± 0.5 (25)	7.4 ± 0.7 (12)	3.9 ± 0.4 (25)	$5.1 \pm 0.2^{**}$ (5)
Ac-RYYRIK-NH ₂ $0.1 \mu\text{M}$	—	—	—	$4.9 \pm 0.7^*$ (5)
Naloxone benzoylhydrazone $5 \mu\text{M}$	$4.2 \pm 0.3^*$ (26)	$5.6 \pm 0.5^*$ (13)	$3.3 \pm 0.3^*$ (21)	9.2 ± 0.7 (5)

The slices or discs were superfused for 110 min with medium containing nomifensine $10 \mu\text{M}$, (–)-sulpiride $3.2 \mu\text{M}$ and naloxone $10 \mu\text{M}$ (slices or discs preincubated with [³H]-dopamine) or desipramine $1 \mu\text{M}$, rauwolscine $1 \mu\text{M}$ and naloxone $10 \mu\text{M}$ (slices preincubated with [³H]-noradrenaline) and, in addition, Ac-RYYRIK-NH₂ or naloxone benzoylhydrazone. Tritium overflow was evoked after 40 (S_1) and 90 min (S_2) of superfusion; the stimulation frequency was 1 and 0.3 Hz for preparations preincubated with [³H]-dopamine and [³H]-noradrenaline, respectively. Means \pm s.e.mean of the number of experiments given in parentheses. * $P < 0.05$, ** $P < 0.001$, compared to the corresponding control.

Table 2 Potencies of N/OFQ, Ac-RYYRIK-NH₂ and naloxone benzoylhydrazone at the NOP receptors examined in the present study^a

	Guinea-pig striatal slices		Mouse striatal slices		Guinea-pig retinal discs	Mouse brain cortex slices	
	<i>pEC</i> ₅₀	<i>App.pA</i> ₂	<i>pEC</i> ₅₀	<i>App.pA</i> ₂	<i>pEC</i> ₅₀	<i>pEC</i> ₅₀	<i>App.pA</i> ₂
N/OFQ	7.9 ^b	—	7.6 ^b	—	8.6 ^b	7.9 ^b	—
Ac-RYYRIK-NH ₂	—	9.1 ^c	—	9.2 ^d	—	8.3 ^e	8.6 ^f
NalBzOH	—	6.8 ^c	—	7.5 ^d	—	—	6.9 ^e

App., Apparent; NalBzOH, naloxone benzoylhydrazone; N/OFQ, nociceptin/orphanin FQ. ^aPotencies were graphically determined from the concentration–response curves shown in Figures 1, 2 and 4. ^bFrom Figure 1. ^cFrom Figure 2A and B. ^dFrom Figure 2C and D. ^eFrom Figure 4B. ^fFrom Figure 4A (mean of the apparent *pA*₂ values for Ac-RYYRIK-NH₂ 0.032 μ M [8.5] and 0.1 μ M [8.7]).

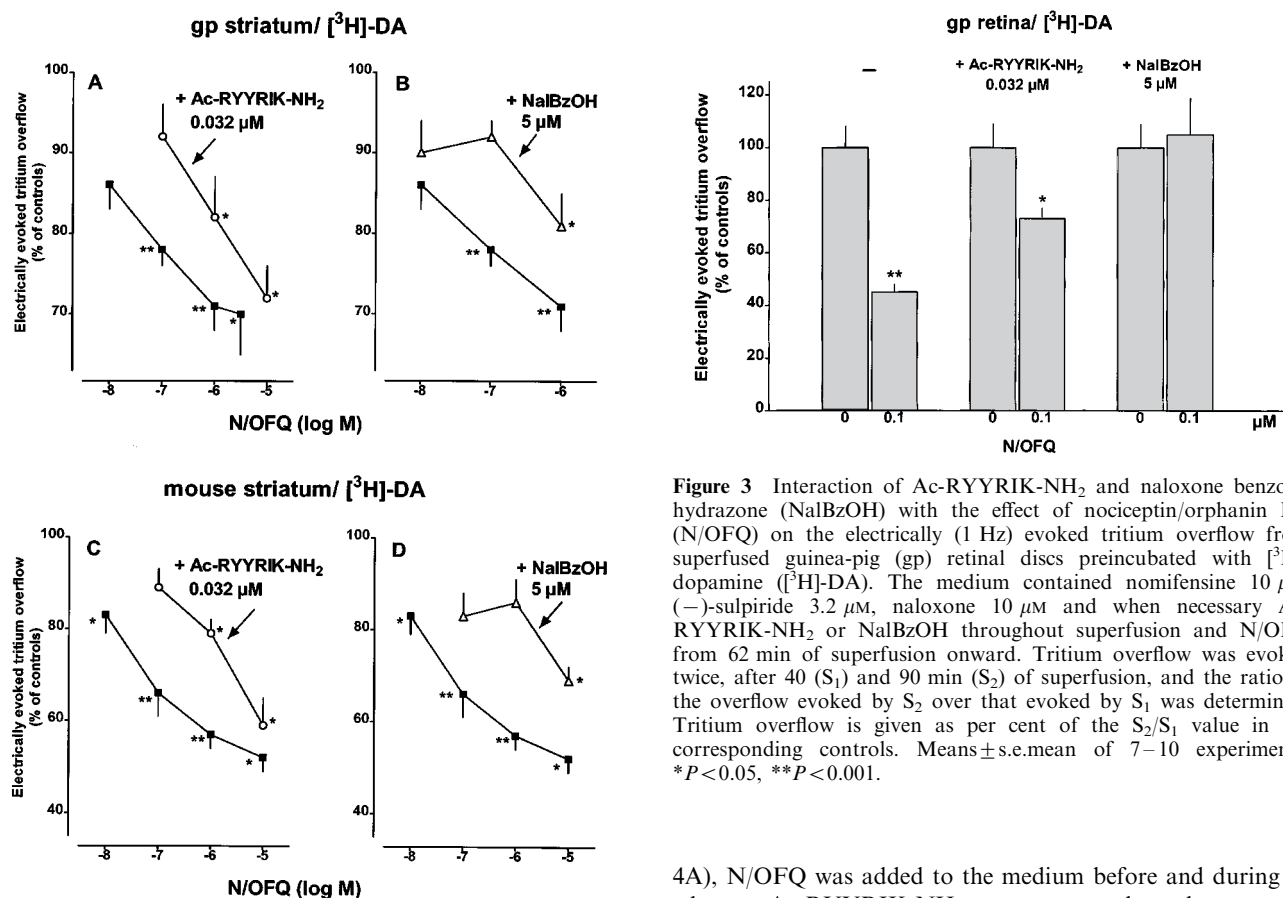


Figure 2 Interaction of Ac-RYYRIK-NH₂ (A, C) and naloxone benzoylhydrazone (NalBzOH) (B, D) with the effect of nociceptin/orphanin FQ (N/OFQ) on the electrically (1 Hz) evoked tritium overflow from superfused guinea-pig (gp) (A, B) and mouse (C, D) striatal slices preincubated with [³H]-dopamine ([³H]-DA). The medium contained nomifensine 10 μ M, (–)-sulpiride 3.2 μ M, naloxone 10 μ M and when necessary Ac-RYYRIK-NH₂ or NalBzOH throughout superfusion and N/OFQ from 62 min of superfusion onward. Tritium overflow was evoked twice, after 40 (*S*₁) and 90 min (*S*₂) of superfusion, and the ratio of the overflow evoked by *S*₂ over that evoked by *S*₁ was determined. Tritium overflow is given as per cent of the *S*₂/*S*₁ value in the corresponding controls (not shown). Means \pm s.e. mean of 4–19 experiments (guinea-pig striatal slices) and 4–15 experiments (mouse striatal slices). **P* < 0.05, ***P* < 0.001.

The effects of N/OFQ, Ac-RYYRIK-NH₂ and naloxone benzoylhydrazone on the evoked tritium overflow were studied in two experimental series. In the first one (Figure

Figure 3 Interaction of Ac-RYYRIK-NH₂ and naloxone benzoylhydrazone (NalBzOH) with the effect of nociceptin/orphanin FQ (N/OFQ) on the electrically (1 Hz) evoked tritium overflow from superfused guinea-pig (gp) retinal discs preincubated with [³H]-dopamine ([³H]-DA). The medium contained nomifensine 10 μ M, (–)-sulpiride 3.2 μ M, naloxone 10 μ M and when necessary Ac-RYYRIK-NH₂ or NalBzOH throughout superfusion and N/OFQ from 62 min of superfusion onward. Tritium overflow was evoked twice, after 40 (*S*₁) and 90 min (*S*₂) of superfusion, and the ratio of the overflow evoked by *S*₂ over that evoked by *S*₁ was determined. Tritium overflow is given as per cent of the *S*₂/*S*₁ value in the corresponding controls. Means \pm s.e. mean of 7–10 experiments. **P* < 0.05, ***P* < 0.001.

4A), N/OFQ was added to the medium before and during *S*₂ whereas Ac-RYYRIK-NH₂ was present throughout superfusion. N/OFQ inhibited the evoked tritium overflow (*S*₂/*S*₁) concentration-dependently; the maximum extent of inhibition amounted to 85% (Figures 1 and 4A). The concentration–response curve of N/OFQ was shifted to the right by Ac-RYYRIK-NH₂ 0.032 and 0.1 μ M (apparent *pA*₂ values of about 8.5; Table 2). Ac-RYYRIK-NH₂ by itself inhibited the electrically evoked tritium overflow (*S*₁) (Table 1).

In the second series of experiments (Figure 4B), Ac-RYYRIK-NH₂ was added to the medium before and during *S*₂ only (like N/OFQ in the first series) whereas naloxone benzoylhydrazone was present in the medium throughout superfusion. Ac-RYYRIK-NH₂ inhibited the electrically evoked tritium overflow (*S*₂/*S*₁) in a concentration-dependent manner. The *pEC*₅₀ value resembles that of N/OFQ (Table 2) whereas the maximum extent of inhibition (by 47%) is markedly lower (compare Figure 4B with 4A), yielding an intrinsic activity relative to N/OFQ of 0.55. The concentra-

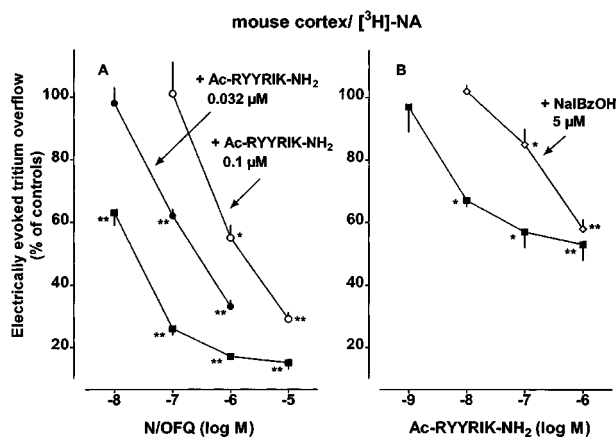


Figure 4 Interaction of Ac-RYYRIK-NH₂ with nociceptin/orphanin FQ (N/OFQ) (A) and of naloxone benzoylhydrazone (NalBzOH) with Ac-RYYRIK-NH₂ (B) in superfused mouse brain cortex slices preincubated with [³H]-noradrenaline ([³H]-NA). The medium contained desipramine 1 μM, rauwolsine 1 μM and naloxone 10 μM. In panel (A), Ac-RYYRIK-NH₂ was present in the medium throughout superfusion whereas N/OFQ was added from 62 min onward. In panel (B), NalBzOH was present in the medium throughout superfusion whereas Ac-RYYRIK-NH₂ was added from 62 min onward. Two periods of electrical stimulation (0.3 Hz) were administered, after 40 (S₁) and 90 min (S₂) of superfusion, and the ratio of the overflow evoked by S₂ over that evoked by S₁ was determined. Tritium overflow is given as per cent of the S₂/S₁ value in the corresponding controls (not shown). Means ± s.e. mean of 4–6 experiments. **P* < 0.05, ***P* < 0.001.

tion-response curve of Ac-RYYRIK-NH₂ was shifted to the right by naloxone benzoylhydrazone 5 μM (for apparent pA₂ value, see Table 2). Naloxone benzoylhydrazone 5 μM by itself did not affect the electrically evoked tritium overflow (S₁) (Table 1).

Discussion

The present study shows that N/OFQ inhibits monoamine release in various tissues *via* NOP receptors. Monoamine release was studied in superfused brain slices or retinal discs and the fact that the electrically evoked tritium overflow in tissues preincubated with [³H]-dopamine and [³H]-noradrenaline is tetrodotoxin-sensitive and Ca²⁺-dependent suggests that we measure quasi-physiological dopamine and noradrenaline release, respectively (Schlicker *et al.*, 1992; present study). Besides naloxone (used to block the classical opioid receptors; see Introduction and below), an inhibitor of the respective uptake mechanism (nomifensine and desipramine) and an antagonist of the respective autoreceptor ((–)-sulpiride and rauwolsine) were added to the medium in order to increase the amount of the evoked tritium overflow. Another reason to block the autoreceptors was our previous finding that rauwolsine increased the extent of NOP receptor-mediated inhibition of noradrenaline release in the mouse brain cortex (Werthwein *et al.*, 1999). The auxiliary drugs do not possess an affinity for NOP receptors since [³H]-N/OFQ binding to mouse brain cortex membranes was not affected (pK_i < 4.5) by naloxone, desipramine, rauwolsine (Werthwein *et al.*, 1999), nomifensine and (–)-sulpiride (unpublished results).

We found that, besides the nigrostriatal, mesolimbic, tuberoinfundibular and incertohypothalamic system (for references, see Introduction), dopaminergic cells in the retina (i.e. amacrine cells; Oh *et al.*, 1999) represent the fifth dopaminergic system affected by N/OFQ. Previous radioligand studies have revealed that NOP receptors occur at high densities in the retina of the rat (85% of the B_{max} in the rat striatum; Makman & Dvorkin, 1997) and the rabbit (25% of the B_{max} in the rabbit brain; Neal *et al.*, 1997). Moreover, N/OFQ has been shown to inhibit the light-induced acetylcholine release in the rabbit retina (Neal *et al.*, 1997). Since only few retinal discs (when compared to the number of striatal slices) could be prepared from the same guinea-pig, only one concentration of Ac-RYYRIK-NH₂ and naloxone benzoylhydrazone was used for the interaction experiments with N/OFQ.

An inhibitory effect of N/OFQ on dopamine release has been shown previously in the striatum of the rat (Shieh & Pan, 2001) and the mouse (Schlicker *et al.*, 1998a) and we show here that such an effect also occurs in the striatum of the guinea-pig. The maximum extent of inhibition in the guinea-pig striatum is lower than that obtained in the mouse striatum. This species difference is reminiscent of the NOP receptor involved in inhibition of cortical noradrenaline release; in this case, again the extent of inhibition in the guinea-pig was lower than that in the mouse (Schlicker *et al.*, 1998b). In order to prove that the effect of N/OFQ in the striatum of either species is mediated *via* NOP receptors we used antagonists. By contrast, Shieh & Pan (2001) had used an antisense oligodeoxynucleotide to prove the involvement of NOP receptors in the N/OFQ-induced inhibition of striatal dopamine release in the rat.

For the sake of comparison, the inhibitory effect of N/OFQ on noradrenaline release in mouse cortex slices was studied as well; the maximum extent of N/OFQ-induced inhibition markedly exceeded that obtained for the other three models. The antagonism of Ac-RYYRIK-NH₂ against N/OFQ confirms that the peptide acts *via* NOP receptors; the same conclusion had been reached in our previous studies in which the less potent and/or less selective antagonists (Phe¹Ψ(CH₂-NH)Gly²]-N/OFQ(1-13)NH₂, naloxone benzoylhydrazone (Schlicker *et al.*, 1998b) and Mr 2266 were used (Bauer *et al.*, 1999).

The apparent pA₂ values for Ac-RYYRIK-NH₂ obtained in three functional models of the present study (range 8.6–9.2) at least equal the pA₂ values reported in the literature for the standard NOP receptor antagonist CompB (Kawamoto *et al.*, 1999) or its racemate, ranging from 7.8 to 8.7 (Bigoni *et al.*, 2000; Chiou & Fan, 2002; Olanas & Onali, 2002; Rominger *et al.*, 2002). On the other hand, Ac-RYYRIK-NH₂ (although not affecting dopamine release in our three models) inhibited noradrenaline release in the mouse cortex by itself. The concentration–response curve of Ac-RYYRIK-NH₂ was shifted to the right by naloxone benzoylhydrazone and the apparent pA₂ value (6.9) was very similar to that obtained in our previous study against N/OFQ (6.6; Schlicker *et al.*, 1998b). Furthermore, the potency of Ac-RYYRIK-NH₂ as an antagonist (apparent pA₂ 8.6) closely resembles its potency as an agonist (pEC₅₀ 8.3), a typical feature of partial agonists. The fact that Ac-RYYRIK-NH₂ is a partial agonist at the NOP receptor in the mouse cortex is not surprising since partial agonistic effects of this compound have been

reported for a variety of NOP receptor models (Berger *et al.*, 2000; Ho *et al.*, 2000; Malinowska *et al.*, 2000b; Mason *et al.*, 2001; Olanas & Onali, 2002).

Interesting enough, naloxone benzoylhydrazone 5 μ M inhibited dopamine release in each of the three models but failed to affect noradrenaline release in the mouse brain cortex at this concentration (present study) and even at 30 μ M (Schlicker *et al.*, 1998b). Therefore, the explanation that this compound, which has previously been shown to behave as a partial agonist at recombinant human NOP receptors (Bigoni *et al.*, 2002), is a partial agonist also at the NOP receptors causing inhibition of striatal and retinal dopamine release is not plausible. Although no further attempts were made to clarify the unexpected findings with naloxone benzoylhydrazone, the possibility has to be considered that this drug inhibited dopamine release due to an agonistic effect on classical opioid receptors. This view is supported by recent results by Bigoni *et al.* (2002), who showed that naloxone benzoylhydrazone 3 μ M inhibited the

electrically induced twitch response of the isolated guinea-pig ileum and that naloxone 30 μ M was necessary to abolish this effect.

In conclusion, our study shows (i) that N/OFQ inhibits dopamine release in the retina, (ii) that this effect and its inhibitory effect on monoamine release in another three models involves NOP receptors and (iii) that Ac-RYYRIK-NH₂ is a very potent antagonist at these receptors although it proves to be a partial agonist at the NOP receptor involved in inhibition of noradrenaline release in the mouse brain cortex.

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