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Comparison of the ORL1 receptor-mediated inhibition of noradrenaline release in human and rat neocortical slices

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1 The effects of nociceptin/orphanin (N/OFQ) and the selective ORL1 antagonist J-113397 (1-[(3R,4R)-1-cyclo-octylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2H-benzimidazol-2-one) were studied on electrically-evoked release of [^{3}H]-noradrenaline ([^{3}H]-NA) from human and rat neocortical slices. Specimens of human tissue were obtained during neurosurgery.

2 Slices were preincubated with 0.1 μM [^{3}H]-NA, superfused in the presence of desipramine, idazoxan, and naloxone (1 μM each), and stimulated electrically up to three times under conditions (4 pulses, 100 Hz, 2 ms, 60 mA) that prevent inhibition of evoked [^{3}H]-NA release by endogenous modulators accumulating during ongoing stimulation.

3 N/OFQ decreased electrically-evoked [^{3}H]-NA release in both human and rat neocortical slices in a concentration-dependent manner. The respective pEC_{50} values were 7.74 [CI_{95}: 7.47, 8.04] and 7.64 [CI_{95}: 7.48, 7.77], and the maximal inhibitions were 36.9% [CI_{95}: 32.4%, 41.8%] and 66.4% [CI_{95}: 61.7%, 72.7%].

4 N/OFQ (1 μM) inhibited K^+ (15 mM)-evoked [^{3}H]-NA release from neocortical slices of both species by a similar magnitude, either in the presence or absence of tetrodotoxin.

5 The nonpeptide ORL1 antagonist J-113397 competitively attenuated, with similar potency, the inhibition of electrically-evoked [^{3}H]-NA release by N/OFQ in both species (pA_2 values: human, 8.16 [CI_{95}: 7.64, 8.64]; rat, 8.47 [CI_{95}: 8.27, 8.67]). J-113397 (0.1 μM) by itself did not alter either the evoked or spontaneous [^{3}H]-NA release, suggesting that presynaptic ORL1 receptors are not activated by endogenous N/OFQ under the stimulation conditions employed.

6 This study provides the first evidence that N/OFQ modulates [^{3}H]-NA release in human neocortex *via* specific ORL1 receptors most likely located on noradrenergic axon terminals.
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Abbreviations: CI_{95}, 95% confidence interval; E_{max}, maximal effect; J-113397, (1-[(3R,4R)-1-cyclo-octylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2H-benzimidazol-2-one); N/OFQ, nociceptin; NA, noradrenaline; pEC_{50}, negative logarithm of the concentration at half-maximal effect; TTX, tetrodotoxin

Introduction

Nociceptin/Orphanin FQ (N/OFQ) is a brain heptadecapeptide identified as the endogenous ligand for the opioid receptor-like 1 (ORL1) receptor (for reviews see: Calo *et al*., 2000; Meunier, 1997; Mogil & Pasternak, 2001; Vlaskovska & Kasakov, 2000). Although this receptor only poorly binds opioid receptor ligands (Mollereau *et al*., 1994) and, conversely, the ORL1 agonist N/OFQ also shows no appreciable affinity to classical opioid receptors (Mogil *et al*., 1996), the effects of N/OFQ at the cellular level are similar to those of opioids (for reviews see: Calo *et al*., 2000; Meunier, 1997; Vlaskovska & Kasakov, 2000).

At the systemic level, administration of N/OFQ or opioid peptides can produce both opposing and similar effects. Thus administration of N/OFQ i.c.v. has been reported to produce either hyperalgesia or functional antagonism of opioid receptor-mediated antinociception, although an antinociceptive effect is more consistently reported for intrathecal administration (Meunier, 1997). In a recent review on the behavioural pharmacology of the N/OFQ ORL1 system (Mogil & Pasternak, 2001), an involvement in the mechanisms of pain, opioid dependence and tolerance, anxiety, fear and stress, as well as in learning and memory was suggested. In all of these conditions a key role of central noradrenergic transmission has been recognized, that is also relevant to human brain.

Since many of the *in vivo* effects of N/OFQ appear to be related to a presynaptic modulation of neurotransmitter

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release (for review see: Schlicker & Morari, 2000), the influence of this peptide on the release of noradrenaline (NA) seems of special interest. Earlier reports have shown that N/OFQ inhibited the *in vitro* release of NA from slices of neocortex, hippocampus, hypothalamus, and cerebellum of mouse, rat, or guinea-pig (Schlicker *et al*., 1998; Trendelenburg *et al*., 2000; Werthwein *et al*., 1999). However, there is a lack of information on the effects of N/OFQ on neurotransmitter release from human brain tissue, even though the existence of this peptide and ORL1 receptors is well established in man (see: Meunier, 1997; Peluso *et al*., 1998).

The first aim of the present study was therefore to assess the effects of N/OFQ on the release of NA from fresh specimens of human brain tissue. In addition, we characterized the interaction of N/OFQ and the recently discovered selective and potent nonpeptide ORL1 antagonist J-113397 (1-[(3R,4R)-1-cyclo-octylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2H-benzimidazol-2-one) (Kawamoto *et al*., 1999; Ozaki *et al*., 2000). Previously, the lack of selective N/OFQ antagonists has been a significant impediment to elucidate the mechanism and receptor specificity of N/OFQ action. Finally, considering possible species differences in the presynaptic effects of N/OFQ (for review see Schlicker & Morari, 2000), we also investigated, under identical conditions, the effects of N/OFQ and J-113397 in the neocortex of rats.

Methods

Tissue preparation

Fresh specimens of human neocortex (i.e., frontal, temporal, parietal or occipital lobe of either left or right hemisphere) were obtained during surgical access to remove epileptic or tumorous tissue from 10 female (aged from 12 to 63 years) and 5 male (aged from 10 to 58 years) patients. This procedure was approved by the local Ethical Committee of the University of Freiburg. The patients were also informed of the purpose of the investigation, and signed a declaration of consent. The surgically removed tissue was immediately immersed into ice-cold oxygenated buffer. Specimens of pure grey matter (about 8×3 mm) were carefully dissected from the underlying white matter, and cut into 350 μm thick slices using a McIllwain tissue chopper (Bachofer, Reutlingen, Germany). Specimens were discarded if visibly infiltrated by tumorous tissue. Microscopic infiltration of tissue by malignant growth could, moreover, also be detected retrospectively, since a significantly lower stimulation-evoked tritium efflux was obtained from such slices; consequently, data from these slices were excluded from further evaluation. Similarly, the neocortical area to be removed in epileptic patients was not used in experiments if it was identified as 'epileptogenic' by presurgical EEG diagnosis. Drugs given to patients before and/or during surgery were as described previously (Jehle *et al*., 2000), and did not appear to modify the evoked release of [^{3}H]-NA.

Neocortical slices were also prepared from male rats (Wistar; 250–350 g). These animals, maintained according to institutional policies and guidelines, were sacrificed by cervical dislocation. The brains were rapidly removed and placed in ice-cold oxygenated buffer. Slices from the parieto-occipital cortex (350 μm thick) were prepared as described above.

Electrically-evoked [^{3}H]-NA release

The slices were incubated at 37°C for 45 min (human) or 30 min (rat) in a modified Krebs-Henseleit buffer (composition (mM): NaCl 121, KCl 1.8, $CaCl_2$ 1.3, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, ascorbic acid 0.6; saturated with 95% O_2/5% CO_2; pH 7.4) containing [^{3}H]-NA (0.1 μM). After incubation, the slices were rinsed, transferred to superfusion chambers (volume 0.1 ml), and superfused at a rate of 0.4 ml min^{-1} with buffer routinely supplemented with desipramine (1 μM), idazoxan (1 μM), and naloxone (1 μM). Collection of 5-min superfusate fractions began after a 50-min precollection period which allowed equilibration of basal tritium outflow. The slices were stimulated electrically (4 pulses, 100 Hz, 2 ms, 60 mA) under conditions which prevent effects of endogenously released transmitters or modulators on the evoked NA release (see Discussion). Field stimulation was applied after 60, 110 and 160 min (S_1, S_2,. S_3). Drugs to be investigated were added to the medium from 20 min before S_2 and S_3 onwards, with increasing concentrations from S_2 to S_3. The concentrations used did not change basal tritium outflow (data not shown). At the end of the experiment, slices were solubilized (0.5 ml Soluene 350; Packard Instruments, Frankfurt, Germany), and radioactivity of both slices and superfusate fractions was determined by liquid scintillation spectrometry after addition of scintillation cocktail (Ultima Gold; Packard Instruments, Frankfurt, Germany).

Potassium-evoked [^{3}H]-NA release

Human and rat neocortical slices were prepared, preincubated with [^{3}H]-NA and superfused at a rate of 0.4 ml/min in chambers (160 μl) with the above buffer containing desipramine, idazoxan, and naloxone. Some of these superfused slices were also exposed to tetrodotoxin (TTX, 0.3 μM). The collection of 18 5-min superfusate fractions began after a 50-min precollection period. The slices were stimulated twice with K^+ (using a 4-way valve) by increasing the K^+ concentration from 3 to 15 mM (osmolarity maintained by decreasing the Na^+ concentration) for an 8-min period after 60 and 110 min of superfusion. The determination of radioactivity in the slices and superfusate fractions was as described above.

Calculations and statistics

The tritium outflow was calculated as a fraction of the tritium content in the slice at the onset of the corresponding collection period (fractional rate). The effects of drugs on basal tritium outflow were evaluated as described previously (Jehle *et al*., 2000). The respective stimulation-evoked tritium overflow at S_1, S_2 and S_3, expressed as a per cent of the tritium content of the slice at the onset of the respective stimulation period, was calculated by subtracting basal outflow. The basal outflow was assumed to decline linearly from 5 min before stimulation to the 5-min period occurring 20–25 min after the onset of each stimulation. The effects of

drugs on the stimulation-evoked overflow were estimated by calculating the ratio S_X/S_1 of the evoked [^{3}H]-NA release at the respective stimulation periods. All drug effects were normalized by dividing each individual S_X/S_1-ratio by the mean ratio of the corresponding controls (no drug addition before S_X). All results are given as means with 95% confidence intervals (CI_{95}) to assess the statistical significance of differences (Altman, 1991; Gardner & Altman, 1986). For estimation of pEC_{50} and E_{max} values of N/OFQ (in the absence or presence of J-113397) from concentration-effect curves, individual S_X/S_1 data (normalized) were evaluated by non-linear regression analysis as described previously (Feuerstein & Limberger, 1999). The apparent pA_2 of J-113397 was then calculated using the formula of Furchgott (Furchgott, 1972): $pA_2 = \log(10^{pEC50\text{-}pEC50^*} - 1) - \log[\text{J-113397}]$, with 'pEC50' being the negative logarithm of the EC50 concentration of N/OFQ, 'pEC50*' the negative logarithm of the concentration of N/OFQ yielding a half-maximal effect in the presence of (0.1 μM) J-113397, and 'log[J-113397]' the logarithm of the antagonist concentration.

Drugs

The substances commercially purchased included L-[ring-2,5,6-^{3}H]-noradrenaline (1.92 TBq/mmol) (NEN; Dreieich, Germany); desipramine hydrochloride, idazoxan hydrochloride, naloxone hydrochloride dihydrate, and tetrodotoxin (Sigma-Aldrich; Deisenhofen, Germany). N/OFQ was acquired from several sources (Peptides International, Louisville, KY, U.S.A.; DRG Instruments, Marburg, Germany; Tocris Cookson, Biotrend Chemikalien GmbH, Köln, Germany). J-113397 (1-[(3R,4R)-1-cyclo-octylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2H-benzimidazol-2-one) was prepared by custom synthesis (Pfizer; Cambridge, U.K.). Stock solutions (1 mg ml^{-1}) were prepared in distilled water (N/OFQ) or ethanol (J-113397); the latter solvent yielded a final concentration in the superfusion medium of 760 nM; none of the solvents given alone affected the basal or evoked overflow of tritium.

Results

Effects of N/OFQ on electrically-evoked [^{3}H]-NA release

Electrical field stimulation of human and rat neocortical slices, preincubated with [^{3}H]-NA, led to an overflow of tritium which represents evoked [^{3}H]-NA release (see discussion). At the first stimulation period (S_1), this evoked release (relative to tissue tritium) was 0.46% (CI_{95}: (0.43%, 0.50%), $n=50$) in human and 1.91% (CI_{95}: (1.83%, 1.99%), $n=92$) in rat neocortical slices. N/OFQ inhibited the electrically-evoked [^{3}H]-NA release from human neocortical slices in a concentration-dependent manner (Figure 1). Nonlinear regression analysis of these data gave a maximal inhibitory effect (E_{max}) of 36.9% (CI_{95}: (32.4%, 41.8%)) and a pEC_{50} value of 7.74 (CI_{95}: (7.47, 8.04)). As shown in Figure 2, N/OFQ produced a more robust inhibition of electrically-evoked [^{3}H]-NA release from rat neocortical slices. The E_{max} value was 66.4% (CI_{95}: (61.7%, 72.7%)). Although this E_{max} value was significantly higher than that from human tissue, the N/OFQ concentration-effect curve was of comparable shape and yielded a similar pEC_{50} value of 7.64 (CI_{95}: (7.48, 7.77)). (Note that the slope parameters (c-values) were near unity as obtained by the two nonlinear regression analyses (for human and rat neocortical slices); consequently, the pEC_{50} values can be considered to represent the corresponding pK_d values (i.e., the dissociation constants of N/OFQ at ORL1 receptors of both species) (Feuerstein & Limberger, 1999)).

Effects of the ORL1 antagonist J-113397 on electrically-evoked [^{3}H]-NA release

The ORL antagonist J-113397 (0.1 μM), added to the superfusion buffer from 20 min before S_2 onwards, altered neither the basal tritium efflux or electrically-evoked [^{3}H]-NA release from both human and rat neocortical slices (Table 1). In pilot experiments, however, higher concentrations of J-113397 (1 and 3.2 μM) were observed to significantly enhance basal tritium efflux from rat neocortical slices (data not shown).

Effect of J-113397 on N/OFQ-mediated inhibition of electrically-evoked [^{3}H]-NA release

In this series of experiments, the ORL1 antagonist J-113397 (0.1 μM) was present throughout superfusion until the end of the experiment. The mean S_1 value was 0.46% (CI_{95}: (0.44%, 0.48%), $n=63$) of tissue tritium in human neocortical slices, and 1.85% (CI_{95}: (1.72%, 1.98%), $n=41$) of tissue tritium in rat neocortical slices; these S_1 values did not differ from those S_1 values in the absence of J-113397. In the presence of J-113397, however, the concentration-effect curve of N/OFQ for human neocortical [^{3}H]-NA release was shifted significantly to the right (Figure 1). The pEC_{50} value, as estimated by nonlinear regression analysis of the data points (assuming the same E_{max} value as in the absence of J-113397), was 6.55 (CI_{95}: (6.07, 6.98)). A pA_2 value of 8.16 ($CI_{95}=7.64$, 8.64)) was determined for J-113397 from the pEC_{50} values in the absence and presence of this antagonist.

In rat neocortical slices, the concentration-effect curve of N/OFQ was also significantly shifted to the right by J-113397 (0.1 μM; Figure 2). The pEC_{50} value, as estimated by nonlinear regression analysis of the data points (assuming the same E_{max} value as in the absence of J-113397), was 6.15 (CI_{95}: (6.00, 6.30)). The pA_2 value at 8.47 (CI_{95}: (8.27, 8.67)) was not significantly different from that determined for human neocortical slices.

Effect of N/OFQ on K^+-evoked [^{3}H]-NA release in the absence or presence of TTX

N/OFQ (1 μM) significantly inhibited K^+-evoked [^{3}H]-NA release from both rat and human neocortical slices (Figure 3). In the presence of TTX (0.3 μM), moreover, this inhibitory effect of N/OFQ was still present, although it appeared to be slightly diminished in human neocortical slices. TTX by itself markedly reduced the evoked tritium overflow at S_1 (see legend to Figure 3).

Table 1 Effects of J-113397 (0.1 μM) on electrically-evoked tritium overflow and basal tritium outflow from human and rat neocortical slices preincubated with [^{3}H]-NA

		Evoked release of [^{3}H]-NA S_2/S_1	*Basal efflux of [^{3}H] b_2/b_1*
Human neocortex:			
Controls	($n=8$)	1.07 (0.89; 1.25)	0.76 (0.68; 0.84)
J-113397	($n=11$)	1.00 (0.90; 1.10)	0.84 (0.76; 0.92)
Rat neocortex:			
Controls	($n=11$)	1.03 (0.97; 1.09)	0.88 (0.83; 0.93)
J-113397	($n=11$)	1.01 (0.93; 1.09)	0.94 (0.90; 0.97)

Human and rat neocortical slices were preincubated with [^{3}H]-NA and then superfused in the presence of desipramine, idazoxan and naloxone (1 μM each). The release of [^{3}H]-NA was induced by two electrical field stimulations (S_1, S_2; four pulses of 2 ms, 60 mA, 100 Hz). J-113397 (0.1 μM) was added to the superfusion buffer from 20 min before S_2 onwards. The effects of this drug on basal tritium outflow are given as the ratio (mean [CI_{95}]) of the fractional rates corresponding to the fractions immediately preceding the simulations; its effects on evoked tritium overflow are presented as the ratio S_2/S_1 (mean [CI_{95}]) of the tritium overflows induced by the corresponding stimulations.

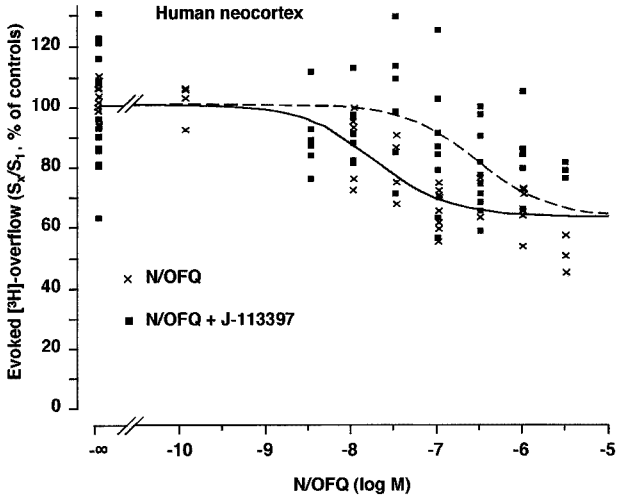

Figure 1 Effects of N/OFQ on electrically-evoked tritium overflow in human neocortical slices. These slices were preincubated with [^{3}H]-NA and then superfused in the presence of despramine, idazoxan and naloxone (1 μM each); in some of the experiments, J-113397 (0.1 μM) was also present throughout superfusion. Release of [^{3}H]-NA was induced by up to three electrical field stimulations (S_1, S_2, S_3; four pulses of 2 ms, 60 mA, 100 Hz). N/OFQ alone (x) or in the presence of J-113397 (0.1 μM) throughout superfusion (■), was added in increasing concentrations before S_2 or S_3 as indicated on the abscissa. Ordinate: evoked overflow of tritium as calculated from S_X/S_1 ratios, expressed as a percentage of the corresponding untreated controls. Data points shown represent single values fitted by a nonlinear regression analysis as described in Methods (for further details see text).

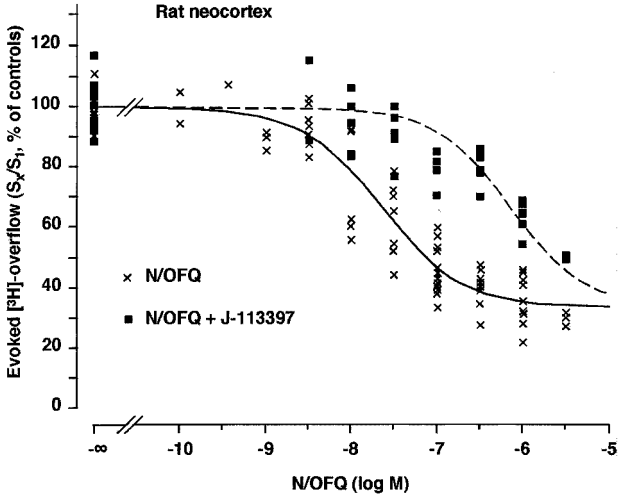

Figure 2 Effects of N/OFQ on electrically-evoked tritium overflow in rat neocortical slices. These slices were preincubated with [^{3}H]-NA, superfused and electrically stimulated (S_1, S_2, S_3) exactly as described in the legend to Figure 1. N/OFQ alone (x) or in the presence of J-113397 (0.1 μM) throughout superfusion (■), was added in increasing concentrations before S_2 or S_3 as indicated on the abscissa. Ordinate: evoked overflow of tritium as calculated from S_X/S_1 ratios, expressed as a percentage of the corresponding untreated controls. Data points shown represent single values fitted by nonlinear regression analysis as described in Methods (for further details see text).

Discussion and conclusions

Preincubation of neocortical slices of the human and rat brain with low concentrations of [^{3}H]-NA (0.1 μM) is generally assumed to preferentially label noradrenergic but not serotonergic axon terminals equally present in this tissue. This assumption is supported by the observation, that the affinity (K_m) of NA for the (human) 5-HT transporter is about 50 times lower than for the corresponding NA transporter (Tatsumi *et al*., 1997). Moreover, the difference in the rate of [^{3}H]-NA uptake by the two different uptake carriers may be even higher, since previous data suggest that also the V_{max} of NA at the 5-HT transporter is much lower than that at the NA carrier (James & Bryan-Lluka, 1997; Paczkowski *et al*., 1996).

Electrically-evoked overflow of tritium from slices of the human and rat neocortex preincubated with [^{3}H]-NA has previously been shown to be Ca^{2+}-dependent and tetrodotoxin-sensitive (Feuerstein *et al*., 1990; Taube *et al*., 1977). Therefore, this evoked tritium overflow is assumed to represent action potential-induced, exocytotic release of NA from noradrenergic axon terminals. Note also that the chosen stimulation conditions (so called 'pseudo one pulses') preclude direct or indirect interference of endogenous transmitters or modulators released during ongoing stimulation with the effects of exogenously added agonists (Singer, 1988).

The present investigation demonstrates for the first time that, consistent with rodent experiments (Schlicker & Morari, 2000), N/OFQ inhibits stimulation-evoked release of [^{3}H]-NA from human neocortex (Figure 1). Since all experiments were performed in the presence of the NA reuptake inhibitor

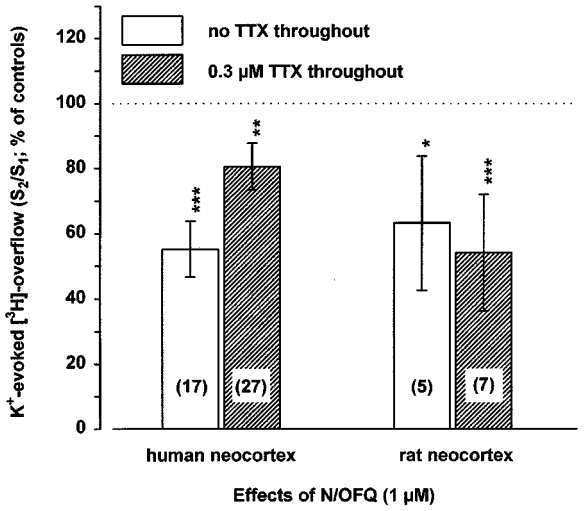

Figure 3 Effects of N/OFQ in the absence or presence of TTX (0.3 μM) on K^+-evoked tritium overflow from human and rat neocortical slices. Stimulation with 15 mM K^+ for 8 min was performed twice (S_1, S_2) in the routine presence of desipramine, idazoxan, and naloxone (1 μM each throughout superfusion). N/OFQ (1 μM) was added to the superfusion buffer from 25 min before S_2 onwards. The effects of N/OFQ on the ratio S_2/S_1 of the evoked tritium overflows are shown (means [CI_{95}], expressed as a percentage of untreated controls; *n*, in parentheses). The tritium overflow at S_1 (mean [CI_{95}]) in the absence and presence of TTX was, respectively, 8.4% (7.4, 9.4%, $n=30$) and 0.8% (0.7, 0.9%, $n=50$) in human neocortical slices, and 6.3% (4.6, 8.1%, $n=11$) and 1.3% (1.1, 1.6%, $n=16$) in rat neocortical slices. Significance of effects *vs* corresponding controls: $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

desipramine, the α_2-adrenoceptor antagonist idazoxan, and the non-selective opioid receptor antagonist naloxone, neither the NA transporter, α_2-adrenoceptors (Allgaier *et al*., 1991; Schlicker & Göthert, 1998) or the classical opioid receptors (Calo *et al*., 2000; Schlicker *et al*., 1998) are involved in the inhibitory effects of N/OFQ. However, the effects of N/OFQ were significantly attenuated by the recently developed specific ORL1 receptor antagonist J-113397 (Kawamoto *et al*., 1999; Ozaki *et al*., 2000). Taken together, these observations lead to the suggestion that N/OFQ acts at specific ORL1 receptors on noradrenergic neurones in human neocortex. Moreover, since the effects of N/OFQ were still present using a stimulation paradigm (K^+ depolarization of slices in the presence of TTX, Figure 3) which prevents action potential propagation, it may be further concluded that these ORL1 receptors are most probably located on the axon terminals themselves of these neurones. In this context it should be noted that the presence of TTX strongly reduced the K^+-evoked overflow of tritium (see legend to Figure 3), indicating that a large part of it was due to an increase of the spontaneous firing rate of noradrenergic neurones. In addition to differences in the duration of the two stimulation paradigms (8 min *vs* 32 ms), this fact may also explain the much larger amount of [^{3}H]-overflow during K^+-stimulation as compared to that during electrical stimulation.

Similar conclusions may also be drawn from the experiments on rat neocortical slices which were performed in parallel (Figures 2 and 3). Although the effects of N/OFQ on rat neocortical NA release have already been described by other groups (Schlicker *et al*., 1998; Siniscalchi *et al*., 1999b), the present study is the first detailed analysis of the potency of N/OFQ and J-113397 on NA release in this *in vitro* neurotransmitter release model. Interestingly, the pEC_{50} values of N/OFQ obtained under the experimental conditions were similar for both rat (7.64) and human neocortex (7.74), and are also comparable to that reported for mouse neocortex (Schlicker *et al*., 1998). These findings suggest that the affinity of N/OFQ for ORL1 receptors is very similar, if not identical, in human, rat, and mouse neocortices.

Regardless of these similarities, there were significant differences in the maximal inhibition of rat and human neocortical [^{3}H]-NA release by N/OFQ; the respective E_{max} values were 64 and 37%. Species differences in the maximal inhibitory effects of N/OFQ have been noted previously: N/OFQ (1 μM) was shown to decrease electrically-evoked [^{3}H]-NA release from neocortical slices by 80% in mice, 71% in rats, and only 36% in guinea-pigs (Schlicker *et al*., 1998).

Several explanations are possible for the lower efficacy of N/OFQ on neocortical NA release in humans and guinea-pigs as compared to rats and mice: (1) There may be differences in receptor densities and/or signal transduction mechanisms; unfortunately, however, although both rat and human neocortex seem to exhibit high levels of ORL1 receptor expression (Florin *et al*., 2000; Peluso *et al*., 1998), there are no reports directly comparing ORL1 receptor densities in these tissues. (2) The endogenous levels of the (functional) N/OFQ antagonist, nocistatin (Okuda-Ashitaka & Ito, 2000), may vary to alter the efficacy of exogenously applied N/OFQ. Although this possibility requires further investigation, there is data indicating substantially higher levels of nocistatin in the human than rat brain (Okuda-Ashitaka & Ito, 2000). (3) The kinetics of desensitization of the ORL1 receptors may differ in human *vs* rat brain; this seems unlikely, however, as a prolonged exposure of the neocortical slices to N/OFQ did not change its inhibitory effect (Siniscalchi *et al*., 1999a; Werthwein *et al*., 1999). (4) A species-dependent pattern of N/OFQ biodegradation could contribute to differences in its E_{max} values on evoked NA release (Montiel *et al*., 1997; Terenius *et al*., 2000; Vlaskovska *et al*., 1999). For example, biodegradation of N/OFQ by human tumour cell lines results in generation of N-terminal fragments 1-13, 1-9 and 1-6 (Vlaskovska *et al*., 1999); such fragments could conceivably interact with another subpopulation of the ORL1 receptor family. The shorter fragments (1-7, 1-6) of N/OFQ have recently been shown, moreover, to antagonize some *in vivo* effects of N/OFQ in the mouse/rat spinal cord (Sakurada *et al*., 1999, 2000; Suder *et al*., 1999). In support of these observations, [F/G]NC(1-13) reduced K^+-evoked [^{3}H]-NA release from human neocortical slices in preliminary experiments with a similar potency as N/OFQ, but also reduced the inhibitory effects of this latter peptide (data not shown).

Another major finding of the present study is the apparent competitive blocking action of the specific ORL1 antagonist J-113397 (Kawamoto *et al*., 1999; Ozaki *et al*., 2000) on electrically-evoked [^{3}H]-NA release from both human and rat neocortical slices. The pA_2 values of J-113397 estimated in this study are in the low nanomolar range (viz., 8.16 and 8.47 for human and rat neocortex, respectively) and similar to values reported for the effect of this compound to antagonize responses to N/OFQ using biochemical endpoints of GTP binding and adenylate cyclase activity (8.9 and 8.2; (Ozaki *et*

al., 2000). Furthermore, the observation that J-113397 given alone did not enhance electrically-evoked [^{3}H]-NA release (Table 1) suggests that, at least under the present experimental conditions, presynaptic ORL1 receptors in both rat and human neocortex are not tonically activated by endogenous N/OFQ. This conclusion is in basic agreement with a previous investigation using naloxone benzoylhydrazone (Schlicker *et al*., 1998), a substance which has, however, poor ORL1 receptor selectivity and much lower affinity (Clark *et al*., 1989).

In conclusion, N/OFQ modulates NA release in the human neocortex *via* specific presynaptic ORL1 receptors presumably located on noradrenergic axon terminals. This is the first demonstration of functional effects of N/OFQ in human brain tissue. These results may be of clinical interest as evidence is accumulating for the possible utility of ORL1 receptor agonists in anxiety and stress disorders (Bertorelli *et al*., 2000). Finally, this study shows that the selective ORL1 receptor antagonist, J-113397, reduces the inhibitory effects of N/OFQ with similar potency in both human and rat neocortex. Such ORL1 receptor antagonists may be relevant in the treatment of drug dependence in humans (see Ciccocioppo *et al*., 2000); J-113397 was recently found to alleviate the withdrawal reaction in morphine-dependent rats (Ueda *et al*., 2000).

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