British Journal of Pharmacology (1997) 120, 1399–1400 © 1997 Stockton Press All rights reserved 0007–1188/97 $12.00

SPECIAL REPORT

Inhibition by nociceptin of the light-evoked release of ACh from retinal cholinergic neurones

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The retina possesses cholinergic amacrine cells which release acetylcholine (ACh) in response to flickering light. Using an eye-cup preparation in anaesthetized rabbits we found that when the retina was exposed to nociceptin, the light-evoked release of ACh was reduced in a concentration-dependent manner (IC_{50} = 100 nM), the maximum effect being 60% inhibition. Opioid receptors were not involved in the inhibitory effect of nociceptin because its action was not blocked by naloxone (1 μM) and furthermore μ-opioids enhanced the light-evoked release of ACh. Using rabbit retina homogenates we found that the retina possessed a substantial number of high-affinity binding sites for [^{3}H]-nociceptin indicating the presence of ORL_I-receptors. Since [des-Phe^1]-nociceptin, which has no affinity for the ORL_I-receptor, had no effect on the light-evoked release of ACh it is unlikely that the action of nociceptin was simply non-specific. We conclude that the inhibitory effect of nociceptin on retinal ACh release involves activation of the ORL_I receptors.

Keywords: Nociceptin; retina; cholinergic neurones; ACh release; ORL_I receptors

Introduction Opioids acting on μ-receptors enhance the light-evoked release of acetylcholine (ACh), apparently by a direct action on the cholinergic neurones (Neal *et al*., 1994). The ORL_I orphan receptor structurally resembles opioid receptors and a heptadecapeptide, nociceptin, has been identified as a probable endogenous ligand (Meunier *et al*., 1995). Immunohistochemical localization studies of the ORL_I receptor in the rat brain have revealed that the receptor has a wide distribution (Anton *et al*., 1996), but it is not known whether the ORL_I receptor is present in the retina.

The present study was undertaken to see whether the retina possesses ORL_I receptors and to investigate the possibility that nociceptin, like, μ-opioids, modulates ACh release from cholinergic amacrine cells.

Methods ACh release from the retina was measured as described previously (Cunningham & Neal, 1983). Briefly, rabbits were anaesthetized with urethane (1.5 g kg^{-1}, i.p.) and an eye-cup was prepared which was filled, for 30 min, with Krebs Ringer bicarbonate containing [^{3}H]-ACh (0.5 μM). After the eye had been washed with fresh medium containing physostigmine (30 μM) a syringe was used to place 0.5 ml of medium in the eye-cup and this was replaced at 5 min intervals. The total radioactivity in the resulting samples was measured by liquid scintillation counting. The retina was stimulated for 5 min periods by flickering light (3 Hz). [^{3}H]-ACh represented 95–100% of the increase in total radioactivity evoked by light flashes. The electroretinogram was recorded to check the viability of the preparation and to assist in identifying the sites of action of drugs applied to the retina (Cunningham & Neal, 1983).

Nociceptin binding Homogenates of rabbit retina and brain were prepared as previously described (Kosterlitz *et al*., 1981). The number of ORL_I-sites was determined with 1.5 nM [^{3}H]-nociceptin (168 Ci mmol^{-1}; Amersham International). For comparison, the number of opioid binding sites was determined with 3 nM [^{3}H]-bremazocine (25.3 Ci mmol^{-1}; New England Nuclear). Non-specific binding was determined with 100 nM unlabelled nociceptin and 1 μM diprenorphine, respectively. The final incubation volume was 1 ml and the samples were incubated for 60 min at 25°C before filtration over GF/B filters.

Results When the retina was stimulated with flickering light (3 Hz) in the absence of drugs (controls) the release of ACh was increased 1.80 ± 0.09 fold (mean $\pm$ s.e.mean, $n=8$) compared with the spontaneous resting release. When the retina was exposed to medium containing nociceptin the light evoked release of ACh was strikingly reduced (Figure 1a). The maximum inhibitory effect of nociceptin (approximately 60%) was produced at a concentration of 1.0 μM and the IC_{50} was 100 nM (Figure 1b). [Tyr^{14}]-nociceptin, an analogue with similar affinity to the ORL_I receptor as nociceptin, also inhibited the light evoked release (Figure 2). In contrast, [des-Phe^1]-nociceptin, which lacks affinity for the ORL_I receptor, had no effect on evoked ACh release (Figure 2) strongly suggesting that the inhibitory action of nociceptin was not merely a non-specific depressant effect. The inhibitory effect of nociceptin on the light evoked release of ACh was clearly not mediated by μ-opioid receptors because (a) naloxone (1 μM) did not affect the action of nociceptin, (b) the μ-agonist [D-Ala^2, MePhe^4, Gly-ol^5]-enkephalin (DAMGO) enhanced the light evoked release of ACh and (c) when the retina was stimulated with flickering light at 10 Hz, the effect of DAMGO was lost whilst the inhibitory effect on nociceptin was unchanged (Figure 2).

Nociceptin (1 μM) had no effect on the b-wave of the e.r.g. (not illustrated) indicating that the site of action of the peptide was not located at the photoreceptors or the synapses between the receptors and the bipolar cells. Binding studies with [^{3}H]-nociceptin revealed that ORL_I receptors in the retina were present at about 25% of the number in rabbit brain (33.6 ± 7.2, mean $\pm$ s.e.mean, $n=5$ and 132 ± 14, $n=8$ *f*mol mg^{-1} protein, respectively). In comparison, [^{3}H]-bremazocine binding indicated a relatively small number of opioid receptors in retina compared with brain (10.4 ± 2.5, $n=5$ and 299 ± 21 *f*mol mg^{-1} protein, $n=8$, respectively).

Discussion The present results demonstrate the presence of ORL_I receptors in the retina and that nociceptin modulates the release of ACh from cholinergic amacrine cells. The site of

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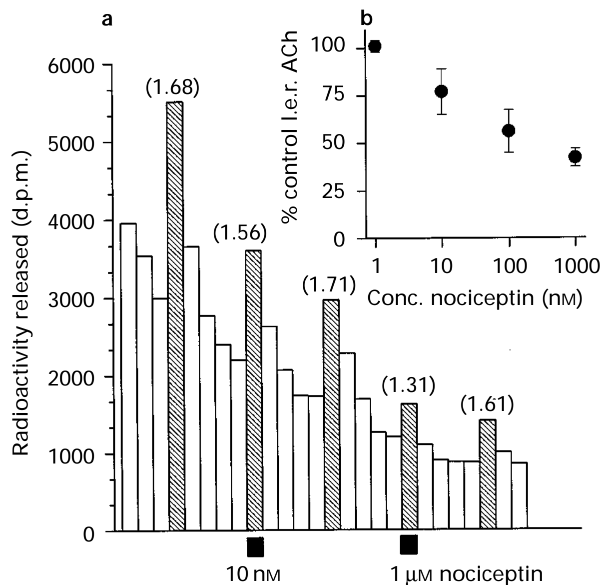

Figure 1 (a) Typical rabbit eye-cup experiment illustrating the inhibitory effects of nociceptin (■) on the light evoked release of ACh. Each column is a 5 min collection period. Hatched columns indicate stimulation with flickering light (3 Hz). Figures in parentheses indicate stimulated release/resting release and are taken as a measure of 'light evoked release of ACh'. (b) Effect of nociceptin concentration on the inhibition of evoked ACh release. The results are expressed as (light evoked release (l.e.r.) in presence of nociceptin/control light evoked release) × 100. Each point is the mean ± s.e.mean of 3–6 separate rabbit experiments. Significant inhibition occurred at nociceptin concentration above 10 nM ($P<0.01$, Student's *t* test).

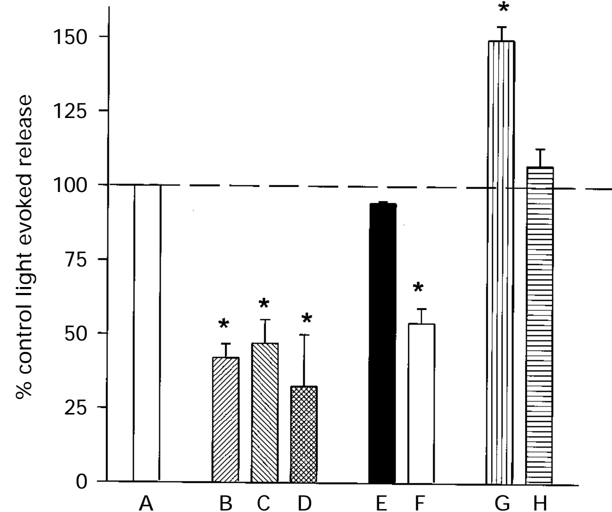

Figure 2 Summary of results illustrating the effects of nociceptin, [des-Phe^1]-nociceptin, [Tyr^{14}]-nociceptin, and DAMGO ([D-Ala^2, MePhe^4, Gly-ol^5]-enkephalin) on the light evoked release of ACh. (A) Control; (B) nociceptin 1 μM, 3 Hz flicker; (C) nociceptin 1 μM, 10 Hz; (D) nociceptin 1 μM + naloxone 1 μM, 3 Hz; (E) [des-Phe^1]-nociceptin; (F) [Tyr^{14}]-nociceptin 1 μM, 3 Hz; (G) DAMGO 10 μM, 3 Hz; (H) DAMGO 10 μM, 10 Hz. Each column is the mean ± s.e.mean of 3–6 separate rabbit experiments. *Indicates significantly different from control ($P<0.01$, Student's *t* test).

action of nociceptin cannot be determined in the present experiments but probably involves synapses in the inner plexiform layer. A reduction in glutamate release from the bipolar cells synapsing with the cholinergic amacrine cells is possible. This action would be consistent with electrophysiological studies in the isolated spinal cord where nociceptin has been shown to inhibit glutamatergic transmission (Faber *et al.*, 1996) and in rat cerebrocortical slices where nociceptin has been found to inhibit glutamate release (Nicol *et al.*, 1996). However, we cannot rule out the possibility that like μ-opioids, nociceptin exerts its inhibitory effect directly on the cholinergic amacrine cells. Patch clamp studies on dissociated rat pyramidal cells have revealed that nociceptin inhibits N-type calcium channels implicated in transmitter release (Knoflach *et al.*, 1996). Thus, in the retina, it is probable that nociceptin produces its effects by inhibiting voltage-dependent calcium channels controlling the release of either glutamate or ACh.

References

ANTON, B., FEIN, J., TO, T., LI, X., SILBERSTEIN, L. & EVANS, C.J. (1996). Immunohistochemical localisation of ORL_1 in the central nervous system of the rat. *J. Compar. Neurol.*, **368,** 229–251.

CUNNINGHAM, J.R. & NEAL, M.J. (1983). Effect of γ-aminobutyric acid agonists, glycine, taurine and neuropeptides on acetylcholine release from the rabbit retina. *J. Physiol.*, **336,** 563–577.

FABER, E.S.L., CHAMBERS, J.P., EVANS, R.H. & HENDERSON, G. (1996). Depression of glutamatergic transmission by nociceptin in the neonatal rat hemisected spinal cord preparation *in vitro*. *Br. J. Pharmacol.*, **119,** 189–190.

KNOFLACH, F., REINSCHEID, R.K., CIVELLI, O. & KEMP, J.A. (1996). Modulation of voltage-gated calcium channels by orphanin FQ in freshly dissociated hippocampal neurons. *J. Neurosci.*, **16,** 6657–6664.

KOSTERLITZ, H.W., PATERSON, S.J. & ROBSON, L.E. (1981). Characterisation of the κ-subtype of the opiate receptor in the guinea-pig brain. *Br. J. Pharmacol.*, **73,** 939–949.

MEUNIER, J.C., MOLLEREAU, C., TOLL, L., SUADEAU, C., MOISAND, C., ALVINERIE, P., BUTOUR, J.L., GUILLEMOT, J.C., FERRARA, P., MONSARRAT, B., MAZARGUIL, H., VASSART, G., PARMENTIER, M. & COSTENTIN, J. (1995). Isolation and structure of the endogenous agonist of opioid receptor-like ORL_1 receptor. *Nature*, **377,** 532–535.

NEAL, M.J., PATERSON, S.J. & CUNNINGHAM, J.R. (1994). Enhancement of retinal acetylcholine release by DAMGO: possibly a direct opioid receptor-mediated excitatory effect. *Br. J. Pharmacol.*, **113,** 789–794.

NICOL, B., LAMBERT, D.G., ROWBOTHAM, D.J., SMART, D. & MCKNIGHT, A.T. (1996). Nociceptin induced inhibition of K^+ evoked glutamate release from rat cerebrocortical slices. *Br. J. Pharmacol.*, **119,** 1081–1083.

(Received January 8, 1997
Accepted February 11, 1997)