Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 540 (2006) 200–201
www.elsevier.com/locate/ejphar

Rapid communication

Schild (apparent pA_2) analysis of a κ-opioid antagonist in *Planaria*

Robert B. Raffa [a,b,*], David A. Baron [a], Ronald J. Tallarida [b]

[a] *Department of Pharmaceutical Sciences, Temple University School of Pharmacy, 3307 N. Broad Street, Philadelphia, PA 19140, USA*
[b] *Department of Pharmacology, Temple University Medical School, 3420 N. Broad Street, Philadelphia, PA 19140, USA*

Received 20 March 2006; received in revised form 23 April 2006; accepted 25 April 2006
Available online 3 May 2006

Abstract

Previous investigators have provided radioimmunological and immunocytochemical evidence for an enkephalinergic (opioid) system in *Planaria* and described naloxone-sensitive qualitative behavioral responses to κ-opioid receptor agonists. We report the application of Schild-analysis to the antagonism of a selective κ agonist (U-50,488H) by a selective κ antagonist (*nor*-BNI) in a quantitative in vivo endpoint. The results provide further evidence of a κ-opioid-like receptor in planarians.

Keywords: κ-opioid receptor; *Planaria*; Schild (pA_2) analysis

Planarians have a centralized nervous system (characterized by cephalic ganglia and spinal processes) and respond in a dose-related and enantiomeric-specific manner to mammalian neurotransmitters. Evidence for the existence of an enkephalinergic system in planarians has come from radioimmunological and immunocytochemical techniques (Venturini et al., 1989). Behavioral responses to high doses (μM) of κ-opioid agonists have also been described (Passarelli et al., 1999). However, to date, no receptor clone, radioligand binding, or more quantitative behavioral data has been reported. In order to provide additional in vivo evidence of a functional κ-opioid receptor in planarians, we applied the Schild-analysis (Arunlakshana and Schild, 1959) to evaluate the antagonism of the selective κ-opioid receptor agonist U-50,488H (*trans*-[*1R,2R*]-3,4-Dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl] cyclohexyl)-benzeneacetamide) by fixed doses of the selective κ-opioid receptor antagonist *nor*-BNI (*nor*-Binaltorphimine). We used our recently devised metric, designated planarian locomotor velocity (*p*LMV) (Raffa et al., 2001), to quantify the planarian behavioral responses.

Planarians (*Dugesia dorotocephala*) were purchased from Carolina Biological Supply Co. (Burlington, NC), acclimated to temperature-controlled (21 °C) laboratory conditions, and tested within 72 h. Each planarian was used only once. U-50,488H methanesulfonate and *nor*-Binaltorphimine were purchased from Sigma Chemical Co. (St. Louis, MO). To measure planarian locomotor velocity, planarians were placed individually into a clear plastic petri dish (14 cm diameter) containing room-temperature (21 °C) tap water treated with AmQuel® water conditioner. The dish was located over graph paper with gridlines spaced 0.5 cm apart. *p*LMV was quantified as the number of gridlines planarians crossed or re-crossed per minute over a 5 min observation period and is expressed as the means (±S.D.) of the cumulative number of gridlines crossed by the planarians per minute. Each planarian was tested individually in one of the following treatments: water only, U-50,488H only, *nor*-BNI only, or U-50,488H plus a fixed-dose of *nor*-BNI (3, 10, or 100 μM).

When tested in water only, planarians displayed a nearly constant *p*LMV of about 14–16 gridlines/min. When tested in the absence of *nor*-BNI, U-50,488H produced a dose-related (1–1000 μM) inhibition of *p*LMV (Fig. 1). Each of the three fixed-doses of *nor*-BNI (3, 10, 100 μM) produced a rightward and parallel shift of the U-50,488H dose-response curve (Fig 1). *Nor*-BNI had no effect of its own on the *p*LMV (data not shown). These results are consistent with the antagonism of U-50,488H by *nor*-BNI, similar to the interaction in mammals.

We applied the pA_2 analysis to these data. The determination of the pA_2 is made from the series of rightward, parallel shifts of the U-50,488H dose-response curve by *nor*-BNI. In this analysis, at any level of effect, the concentrations of agonist are denoted *A* in

* Corresponding author. Department of Pharmaceutical Sciences, Temple University School of Pharmacy, 3307 N. Broad Street, Philadelphia, PA 19140, USA.
E-mail address: robert.raffa@temple.edu (R.B. Raffa).

0014-2999/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2006.04.027

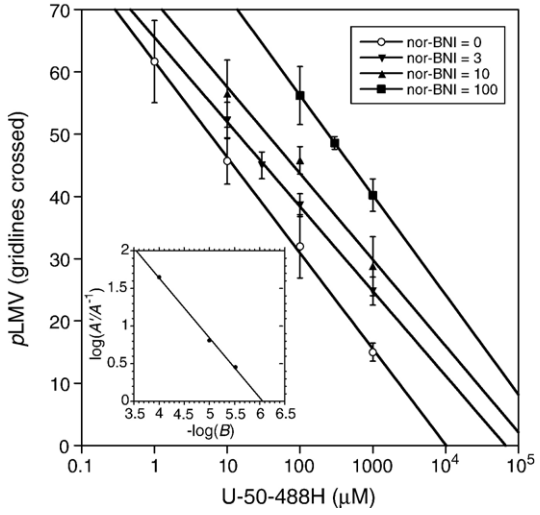

Fig. 1. Rightward, parallel shifts of the U-50,488H dose-response curve by three doses of *nor*-BNI (3, 10 and 100 μM). *p*LMV is expressed as the mean±S.D. cumulative number of gridlines crossed by planarians over a 5 min observation period. N=5 to 10 planarians per point. *Inset*: 'Schild-plot' of the data: shift ratios of the agonist (A) dose-response curves plotted against the corresponding antagonist (B) concentration.

the absence of antagonist and A' in the presence of concentration of antagonist B (in molar units). Applying the law of mass action leads to

$$(A'/A)-1 = B/K_B, \quad (1)$$

where A'/A is the agonist dose-ratio for each concentration B and K_B is the dissociation constant of the antagonist. The pA_2 is defined (Arunlakshana and Schild, 1959) as the negative common logarithm of the concentration B that produces a dose-ratio equal to 2. Eq. (1) is equivalent to

$$\log(A'/A-1) = (\log B)-\log K_B. \quad (2)$$

A 'Schild-plot' is produced by plotting log $(A'/A-1)$ against $-\log B$. For a receptor-mediated effect and competitive interaction between the agonist and antagonist for the same receptor, the results plot as a straight line and the x-intercept estimates the pA_2 of the antagonist and, if the slope$=-1$, the K_B of the antagonist (Tallarida, 2000). In the present case, the data graphed as a straight line ($y=-0.796x+4.82$; $r=0.999$) with the intercept on the horizontal axis (apparent (in vivo) pA_2) of 6.05±0.07 (95% confidence interval: 5.20–6.92), which yields an apparent *nor*-BNI $K_B=8.91\times10^{-7}$ M (95% confidence interval: $1.20-63.0\times10^{-7}$ M). The pA_2 value obtained for *nor*-BNI in the present study, 6.05, is close to the 6.5 reported for *nor*-BNI against enadoline in the mouse tail-flick test (Aceto et al., 2003). The lower affinity of an opioid in an invertebrate compared to a mammal is consistent with previous observations (Stevens, 2003).

Interestingly, despite the excellent straight-line shifts of the U-50,488H dose-response curves by *nor*-BNI, the slope of the Schild-plot of the data was −0.79 (±0.04) rather than −1. Although the confidence interval is wide (−1.29 to 0.30) and includes −1, the large r suggests that −1 is not a likely value, although it cannot be ruled out from the current data. A slope$\neq1$ would be obtained if the ligands interact in other than a 1:1 ratio (Gaddum, 1957). The results would also be expected if the ligands were not as selective for a single opioid receptor subtype in planarians as they are in mammals. Based on the phylogenetic relationships of both the complete receptor sequence and the extracellular domains and the observed nonselectivity of 'selective' opioid antagonists in amphibian models, it has been hypothesized that amphibian opioid receptors are less selective than mammalian opioid receptors (Stevens, 2003). It seems reasonable to assume that this decreased selectivity would extend to invertebrates. This possibility is presently being explored.

In summary, the results of the present study suggest: (1) that *nor*-BNI acts as a pharmacologic antagonist of U-50,488H in *Planaria*, (2) that they interact at a common receptor, and (3) that the common receptor is, functionally, the planarian equivalent of the mammalian κ-opioid receptor.

Acknowledgements

The authors thank Timothy Shickley, PhD. for suggesting *Planaria* as a test model. This work was supported by Grants R01-DA15378 (R.B.R.) and R01-DA09793 (R.J.T.) from the NIH (NIDA).

References

Aceto, M.D., Bowman, E.R., Harris, L.S., Hughes, L.D., Kipps, B.R., May, E.L., 2003. Dependence studies of new compounds in the rhesus monkey, rat and mouse (2002). NIDA Res. Monogr. 183, 191–227.

Arunlakshana, O., Schild, H.O., 1959. Some quantitative uses of drug antagonists. Brit. J. Pharmacol. 14, 48–58.

Gaddum, J.H., 1957. Theories of drug antagonism. Pharmacol. Rev. 9, 211–218.

Passarelli, F., Merante, A., Pontieri, F.E., Margotta, V., Venturini, G., Palladini, G., 1999. Opioid–dopamine interaction in *Planaria*: a behavioral study. Comp. Biochem. Physiol. C 124, 51–55.

Raffa, R.B., Holland, L.J., Schulingkamp, R.J., 2001. Quantitative assessment of dopamine D2 antagonist activity using invertebrate (*Planaria*) locomotion as a functional endpoint. J. Pharmacol. Toxicol. Methods 45, 223–226.

Stevens, C.W., 2003. Opioid research in amphibians: a unique perspective on mechanisms of opioid analgesia and the evolution of opioid receptors. Rev. Analg. 7, 69–82.

Tallarida, R.J., 2000. Drug Synergism and Dose-Effect Data. Chapman & Hall/CRC Press, Boca Raton, FL.

Venturini, G., Carolei, A., Palladini, G., Margotta, V., Lauro, G.M., 1989. Radioimmunological and immunocytochemical demonstration of met-enkephalin in *Planaria*. Comp. Biochem. Physiol. C 74, 23–25.