Upsala J Med Sci 78: 150–152, 1973

Stereospecific Uptake of Narcotic Analgesics by a Subcellular Fraction of the Guinea-pig Ileum

A Preliminary Communication

LARS TERENIUS

From the Department of Pharmacology, University of Uppsala, Uppsala, Sweden

ABSTRACT

Longitudinal muscle of guinea-pig ileum was homogenized. Precipitates from centrifugation were incubated with labelled dihydromorphine and competitors. The uptake of label to a high-speed fraction was stereospecific (the analgesics levorphanol and levo-methadone were more active competitors than their inactive dextro-counterparts). Morphine, normorphine, dextromoramide, the antagonists nalorphine and naloxone inhibited uptake. Codeine was inactive.

INTRODUCTION

The mode of action of narcotic analgesics remains obscure in spite of the intense work which has been attributed to the problem. It is even largely unknown at which site(s) in the CNS they exert their analgesic action. Therefore, the longitudinal muscle of the guinea-pig ileum, which is anatomically well-defined and which is affected by morphine-like analgesics *in vitro* (Schaumann, 1957; Weinstock, 1971) has considerable interest as a model tissue.

The present communication analyses the interaction between narcotic analgesics and subcellular fractions of the longitudinal muscle from guinea-pig ileum. Evidence is provided that there is a specific interaction. Part of this work has been presented at the Scandinavian Pharmacological Society Meeting, Uppsala, June 1972 (Terenius, 1972).

MATERIALS AND METHODS

Guinea-pigs of either sex weighing at least 450 g were killed by a blow on the head. In a typical experiment (12–18 incubation flasks, see below) 5 animals were used. The ileum except for 10–15 cm of the terminal part was removed. Longitudinal muscle strips were prepared from 10–15 cm segments by the method of Rang (1964), carefully avoiding mesenteric fat. The muscle with adhering nerve plexi was finely cut with a pair of scissors. All subsequent operations were at 0° to 4°C. Ten times the weight of ice-cold 0.32 M sucrose in bidistilled water was added. Homogenization was done with an Ultraturrax (Kinematica, Luzern) 2 times for 15 sec at setting 2 with cooling for 2 min inbetween. The homogenate was further disintegrated in a glass homogenizer with a loose-fitting Teflon pestle at low speed. It was then centrifuged at $2 \times 1\,000$ *g* for 10 min. The supernatant was saved and centrifuged at 120 000 *g* for 30 min. In a few experiments the $2 \times 1\,000$ *g* supernatant was centrifugated at 10 000 *g* for 30 min, see text. The respective pellets were resuspended in a small volume of 0.32 M sucrose. Aliquots of the suspension containing about 1 mg protein were added to 4 ml of incubation buffer, NaCl 124 mM, KCl 5 mM, KH_2PO_4 1.2 mM, $CaCl_2$ 0.75 mM, $MgSO_4$ 1.3 mM, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid "HEPES" 26 mM, pH 8.0 at 25°C; this buffer is modified from Bradford & Thomas (1969) which in appropriate cases also contained non-labelled test substances. After a pre-incubation period of 10 minutes at 25°C with the test substances, dihydromorphine-^{3}H (specific activity 66.6 mCi/mg, radiochemical purity 95–98%) in 0.2 ml incubation buffer was added, and the incubation was continued for 1 hour. The incubation was stopped by cooling the vessels on ice. The content was quantitatively transferred to centrifuge tubes and centrifuged for 30 min at 120 000 *g*. Aliquots (0.5 ml) of the supernatant were removed for radioactivity measurement. The remaining supernatant was discarded, and the pellets were rapidly washed with ice-cold buffer. The tubes were immediately inversed and allowed to stand in an in-

Table I. *Competition experiment showing the stereospecificity of the uptake of labelled dihydromorphine to the 120 000 g particulate fraction (Expts. 1–2) or the 10 000 g particulate fraction (Expts. 3–4) of guinea-pig ileum*

The concentration of dihydromorphine was 0.55×10^{-9} M, of levorphanol, dextrorphan and of the methadone antipodes 1.1×10^{-8} M. Each experiment represents one homogenate, each treatment was run in duplicate

	Uptake (DPM/mg protein)							
Competitor	Expt. 1		2		3		4	
None	761	844			1 118	1 252		
Levorphanol	330	346			807	810		
Dextrorphan	653	674			1 052	1 064		
None	647	759	650	634	1 019	1 056	999	1 003
Levo-Methadone	432	424	483	436	665	736	619	626
Dextro-Methadone	565	765	707	742	985	997	847	920

versed position for at least one hour. The pellets were digested with Soluene® (Packard, Downers Grove, Ill.) and the radioactivity content measured. Protein was determined according to Lowry et al. (1951) using bovine serum albumin as standard.

RESULTS

Competition experiments were performed in order to establish the specificity of the interaction between dihydromorphine and the high-speed subcellular fraction. It was found that the optical antipodes, levorphanol/dextrorphan and levo-/dextro-methadone differentially inhibited the uptake of dihydromorphine to the high-speed precipitate, the analgetically more active levo-antipodes being the more active competitors (Table I, expts. 1 and 2). In experiments 3 and 4 (Table I) is shown that the stereospecific uptake is also present in the 10 000 *g* precipitate.

Other narcotic analgesics (morphine, normorphine and dextromoramide) and the narcotic antagonists (nalorphine and naloxone) were active competitors in the test system while codeine was without effect (Table II).

DISCUSSION

Morphine-like compounds inhibit the contractions of the longitudinal muscle of the guinea-pig ileum (see Schaumann, 1957; Weinstock, 1971). This tissue should therefore contain morphine receptors

Table II. *Effect of various analgesics and analgesic antagonists on the uptake (DPM/mg protein) of labelled dihydromorphine (concentration 0.7×10^{-9} M) to a high-speed particulate fraction of guinea-pig ileum*

A different homogenate was used for each substance. Means ±S.E.M. are given for 3 samples per group

	Concentration (M) of competitor			
Substance	0	10^{-9}	10^{-8}	10^{-7}
Morphine	981±54	794±50	585±3	508±55
Dextromoramide	953±46	1 012±29	845±34	615±37
Normorphine	1 069±16	905±6	798±20	658±48
Nalorphine	1 296±42	1 027±44	951±34	570±24
Naloxone	1 121±31	805±32	571±32	395±13
Codeine	1 256±12	—	1 225±18	1 219±62

and an interaction with such receptors might be observable in simple incubation experiments. In view of the very strict steric requirements for activity on the ileum (Schaumann et al., 1953) (which seem identical to those for analgesic activity) one might expect a similar selectivity in the binding properties of the receptor.

The present work shows a stereoselective interaction with the opiates levorphanol and dextrorphan as competitors and as well with the all synthetic, structurally completely different methadone antipodes. Those isomers which are more potent on the ileum (Schaumann et al., 1953) are the more active competitors (Table I). Also another synthetic analgesic, dextromoramide, showed considerable competitive activity. On the other hand, codeine is practically inactive as a competitor (Table II) as on the ileum preparation (Kosterlitz & Watt, 1968). All this evidence favors the assumption that the observed interaction is of fundamental importance for the action of morphine-like drugs and that it could be ascribed to an interaction with a receptor.

It is also notable that the narcotic antagonists, nalorphine and naloxone, are active as competitors (Table II). Although most narcotic antagonists including nalorphine have agonistic properties, naloxone is held to be a pure antagonist (also on the ileum preparation, Kosterlitz & Watt, 1968) indicating that the antagonistic activity of narcotic antagonists may depend on competition with the analgesics for the receptors. Also normorphine is active as competitor (Table II), which is of interest since this compound has been implicated

in the action of morphine (Beckett et al., 1956).

Preliminary experiments with further fractionation (combined with enzymological and morphological examination) indicate that particles rich in acetylcholinesterase and 5′-nucleotidase and not mitochondria are responsible for the uptake.

ACKNOWLEDGMENT

Technical assistance was given by Mrs Ulla Stääv. Normorphine was kindly donated by Dr E. L. May at N.I.H., Bethesda, Md, USA, naloxone by Dr H. W. Kosterlitz, University of Aberdeen, Scotland, and levorphanol and dextrorphan by Hoffmann-La Roche, Basel, Switzerland. The work was supported by the Swedish Medical Research Council (Proj. No. K72-14X-3756-01).

REFERENCES

1. Beckett, A. H., Casy, A. F. & Harper, N. J.: Analgesics and their antagonists: some steric and chemical considerations. J Pharm Pharmacol *8:* 874, 1956.
2. Bradford, H. F. & Thomas, A. J.: Metabolism of glucose and glutamate by synaptosomes from mammalian cerebral cortex. J Neurochem *16:* 1495, 1969.
3. Kosterlitz, H. W. & Watt, A. J.: Kinetic parameters of narcotic agonists and antagonists, with particular reference to N-allylnoroxy-morphone (Naloxone). Brit J Pharmacol *33:* 266, 1968.
4. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. & Randall, R. J.: Protein measurement with the Folin phenol reagent. J Biol Chem *193:* 265, 1951.
5. Rang, H. P.: Stimulant actions of volatile anaesthetics on smooth muscle. Brit J Pharmacol *22:* 356, 1964.
6. Schaumann, O.: Morphin und morphinähnlich wirkende Verbindungen. In Handbuch der experimentellen Pharmakologie, Ergänzungswerk (ed. O. Eichler & A. Farah), vol. 12, pp. 170–189. Springer, Berlin, 1957.
7. Schaumann, O., Jcchum, K. & Schaumann, W.: Analgetika und Darmmotorik. II. Wirkung auf den Längsmuskeltonus. Arch Pharmakol *217:* 360, 1953.
8. Terenius, L.: Specific uptake of narcotic analgesics by subcellular fractions of the guinea-pig ileum. Acta Pharmacol Toxicol *31:* Suppl. I, 50, 1972.
9. Weinstock, M.: Sites of action of narcotic analgesic drugs: in peripheral tissues. *In* Narcotic Drugs, Biochemical Pharmacology (ed. D. H. Clouet), pp. 394–407. Plenum Press, New York.

Received December 15, 1972

Address for reprints:

L. Terenius
Department of Pharmacology
University of Uppsala
Box 573, 751 23 Uppsala
Sweden