Contribution of 'receptor' affinity to analgesic potency

Pharmacokinetic differences will probably be considerable for the chemically heterogeneous group of agents having morphine-like activity. For instance, the partition coefficients heptane/water range from less than 0·0001 for the hydrophilic morphine and normorphine to 100 for the lipophilic methadone and fentanyl (von Cube, Teschemacher & others, 1970). One would expect the lipophilic drugs to cross the blood-brain barrier more easily than the hydrophilic drugs. This was confirmed by whole-body autoradiography with the narcotics dihydromorphine and fentanyl and the narcotic antagonist M5050 which are all physicochemically different. Other pharmacokinetic factors also have to be considered (Appelgren & Terenius, 1973). It is therefore not possible to estimate the relative contribution of receptor affinity to the analgesic activity from classical bioassays involving systemic injection.

A way of reducing the complexity of the *in vivo* system is direct injection of drugs in close proximity to the receptors in the cns. Work by Herz, Albus & others (1970) has revealed sensitive areas close to the 3rd and 4th ventricles of rabbit brain. An intraventricular injection will therefore deliver the drug close to the receptor area and thereby circumvent processes going on outside the brain. However, as discussed below, only by the actual measurement of receptor affinities will it be possible to define the contribution of receptor affinity to the analgesic potency.

The present author has recently described a specific "receptor" for narcotic analgesics in the guinea-pig ileum and in rat brain. The "receptor" has the high structural specificity anticipated for the actual narcotic receptor (Terenius, 1972, 1973 a, b, c). In the present work, the affinities of this "receptor" for a number of narcotic drugs, also tested by intraventricular injection by Kutter, Herz & others (1970) and by autoradiography by Appelgren & Terenius (1973), have been measured.

The experimental procedure is described in detail elsewhere (Terenius, 1973 c). Briefly, a fraction enriched in synaptic plasma membranes (SPM) is prepared from rat brain (except cerebellum). Aliquots of the SPM fraction are incubated with tritium-labelled dihydromorphine (spec. act. 59 Ci mmol^{-1}, at 0·5 × 10^{-9}M) and with various non-labelled competitors for 40 min at 25°. The incubation is terminated by centrifugation in a Beckman Microfuge and the content of radioactivity in the SPM pellet and in the medium is measured. Each competitor is tested at least twice at

Table 1. *"Receptor" affinity and analgesic activity of narcotic analgesics and M5050.*

Substance	"Receptor" affinity IC50 Absolute (nM)	Relative	Analgesic activity[1] Intraventricular injection ED10 Absolute (nmol)	Relative	Intravenous injection ED50 Absolute (nmol)	Relative
(±)-Methadone	7	2·7	1800	0·07	10 800	0·07
Fentanyl	3	6·3	13	10	114	7
Pethidine	600	0·03	4300	0·03	37 100	0·02
Etorphine	0·19	100	1·3	100	8	100
Levorphanol	3	6·3	380[2]	0·3	3790	0·2
Dihydromorphine	3	6·3	74	1·8	42 100	0·02
Morphine	5	3·8	53	2·4	49 500	0·02
Normorphine	15	1·3	160	0·8	460 400	0·002
M5050*	0·18	105				

[1] Kutter & others (1970).

[2] Estimated from a graph (von Cube & others, 1970).

* *N*-cyclopropylmethyl-16,14-endoetheno-7α(1-hydroxy-1-methylethyl)tetrahydronororipavine.

5 concentrations in each run. At each concentration, 3 parallel tubes are run. The specific dihydromorphine binding is taken as the part of the total binding saturated by 10^{-6}M unlabelled drug (Terenius, 1973 c). The inhibitory activity of the competitors is then expressed in percent of specific binding (= the uncompleted value corrected for nonspecific binding). IC50 values are estimated graphically.

Table 1 summarizes the findings. The first four drugs and M5050 can be classified as lipophilic (k heptane–water >10) whilst the others are hydrophilic (k heptane–water $<0{\cdot}01$) (cf. von Cube & others, 1970). The affinities of the various analgesics for the narcotic "receptor" are compared with the analgesic activities of the drugs following intravenous or intraventricular injections in rabbits (Kutter & others, 1970). It is clear that the relative receptor affinities correlate much better with relative analgesic potencies after intraventricular injection than after intravenous injection.

The most notable exception is methadone, which is comparatively less active following intraventricular administration than in "receptor" affinity. Very little is known about the distribution of methadone after intraventricular administration. One possible explanation for this unexpectedly low activity could be the local formation of metabolites, some of which are apparently irreversibly bound to cellular constituents in brain (Misra & Mulé, 1972). A factor which might affect the disposition of the drugs differently even after intraventricular injection could be the transport systems, one of which at least is known to handle morphine. For instance, Asghar & Way (1970) have shown that morphine is actively removed from the cerebroventricular spaces after ventricular perfusion. Carrier-mediated processes in the isolated choroid plexus (Takemori & Stenwick, 1966; Hug, 1967) and from blood to the csf (Wang & Takemori, 1972) have also been described. The relative importance of such processes for the distribution of morphine is inadequately known, and information for other narcotic analgesics is lacking.

In parallel with the present work, other work concerning the narcotic receptor has been reported (Pert & Snyder, 1973 a, b; Simon, Hiller & Edelman, 1973). However, the other groups have used total homogenates of brain for affinity measurements. Since such homogenates are more heterogeneous, interference from non-specific binding to irrelevant biomaterial and metabolic processes may be more critical. Despite this and other differences in experimental approach, the relative binding affinities are grossly similar (Table 2). It should be noted that the absolute values differ considerably, particularly from those of Simon & others (1973). Generally, the present investigation gives higher affinities. This is explainable since the other groups use the labelled indicator drug at much higher concentrations than I do.

Table 2. *Displacement potency of non-labelled drugs on the specific binding of tritium-labelled narcotics. IC50, concentration giving 50% displacement.*

	IC50 (nM) according to various authors		
Nonlabelled agent	Pert & Snyder (1973 b)[1]	Simon & others (1973)[2]	Terenius (this work)[3]
(±)-Methadone	30	300	4
Etorphine	0·3	3	0·2
Levorphanol	2	20	3
Dihydromorphine			3
Morphine	7	200	5
Naloxone	10	30	1

[1] Labelled drug naloxone, uniformly labelled, 6·1 Ci mmol^{-1}, 5nM concentration.

[2] Labelled drug etorphine, uniformly labelled, 3·3 Ci mmol^{-1}, 3nM concentration.

[3] Labelled drug dihydromorphine, specifically labelled, 59 Ci mmol^{-1}, 0·5nM concentration.

This gives too low values for apparent affinity.

The present data on "receptor" affinities support the main conclusion reached by Kutter & others (1970) that lipophilic analgesics more easily cross the blood-brain barrier and get access to the biophase. Thus the extreme potency of etorphine (1000–10 000 × morphine) can to a large extent be explained by this factor.

This work was supported by the Swedish Medical Research Council.

Department of Medical Pharmacology,
Box 573, 751 23, Uppsala, Sweden.

L. TERENIUS

October 9, 1973

REFERENCES

APPELGREN, L.-E. & TERENIUS, L. (1973). *Acta physiol. scand.*, **88**, 175–182.
ASGHAR, K. & WAY, E. L. (1970). *J. Pharmac. exp. Ther.*, **175**, 75–83.
HERZ, A., ALBUS, K., METYŠ, J., SCHUBERT, P. & TESCHEMACHER, H.-J. (1970). *Neuropharmacology*, **9**, 539–551.
HUG, Jr, C. C. (1967). *Biochem. Pharmac.*, **16**, 345–359.
KUTTER, E., HERZ, A., TESCHEMACHER, H.-J. & HESS, R. (1970). *J. mednl Chem.*, **13**, 801–805.
MISRA, A. L. & MULÉ, S. J. (1972). *Nature*, **238**, 155–156.
PERT, C. B. & SNYDER, S. H. (1973a). *Science*, **179**, 1011–1014.
PERT, C. B. & SNYDER, S. H. (1973b). *Proc. nat. Acad. Sci. USA*, **70**, 2243–2247.
SIMON, E. J., HILLER, J. M. & EDELMAN, I. (1973). *Ibid.*, **70**, 1947–1949.
TAKEMORI, A. E. & STENWICK, M. W. (1966). *J. Pharmac. exp. Ther.*, **154**, 586–594.
TERENIUS, L. (1972). *Acta pharmac. tox.*, **31**, *Suppl.* 150.
TERENIUS, L. (1973a). *Ibid.*, **32**, 317–320.
TERENIUS, L. (1973b). *Ibid.*, **33**, 377–384.
TERENIUS, L. (1973c). *Ibid.*, **33**, in the press.
VON CUBE, B., TESCHEMACHER, H.-J., HERZ, A. & HESS, R. (1970). *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **265**, 455–473.
WANG, J. H. & TAKEMORI, A. E. (1972). *J. Pharmac. exp. Ther.*, **183**, 41–48.

Permeability of films of ethyl cellulose and PEG to caffeine

Film coating by polymers is a method for producing sustained-release preparations (Munden, DeKay & Banker, 1964; Nessel, DeKay & Banker, 1964; Kleber, Nash & Lee, 1964; Lappas & McKeehan, 1965). A factor determining the suitability of a film for development of a sustained-release preparation of a drug is the permeability of the film to the drug. Other considerations are the physical properties, stability and toxicity of the film material. Providing drug dissolution is not the rate controlling factor, the release rate of medicinal substances from polymers which are soluble in the digestive system is determined both by the permeability of the film and the rate of dissolution of the polymer (Stempel, 1966), while permeability is the only factor governing the rate of release through a polymer which is insoluble in the digestive system. An added advantage of the insoluble type of film is that it is not absorbed by the body.

Ethyl cellulose is an example of a non-toxic polymer which is insoluble in the digestive system. Experiments in which the permeability of an ethyl cellulose film was increased by the addition of cellulosic polymers were carried out by Fites, Banker & Smolen (1970), Coletta & Rubin (1964) and Shah & Sheth (1972). We have examined the effect of using a water-soluble polyethylene glycol of high molecular weight (4000) as additive.

Membranes were prepared by the techniques of Munden, DeKay & Banker (1964)