In conclusion, the osmotic minipump provides a useful tool for the study of steady-state kinetics of drug distribution and metabolism. A constant *in vitro* release rate by these minipumps is reflected in constant plasma concentrations of this drug and its metabolites throughout the infusion period. Without disturbing the experimental animal for frequent injections a steady-state distribution can thus be accomplished.

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The effects of some analgesic and neuroleptic drugs on the spasmogenic actions of substance P on guinea-pig ileum

J. M. ELLIOTT*, J. B. GLEN†, *Biology Department, ICI Pharmaceuticals Division, Alderley Park, Cheshire, U.K.*

The role of substance P (SP) as a neurotransmitter particularly associated with pain sensation at the level of the spinal cord is gradually gaining support (Henry, 1976; Randic & Miletic, 1977). Substance P appears to be stored in vesicles (Cuello, Jessel & others, 1977) and can be released from nervous tissue following depolarization (Iversen, Jessel & Kanazawa, 1976; Otsuka & Konishi, 1976). Inhibition of release of SP from the trigeminal nucleus by opiates and by endorphins (Jessel & Iversen, 1977) suggests that the actions of SP and the endogenous opiates are closely linked to pain perception. However, some reports claim that the action of SP may not be mediated by a unique receptor but possibly via an opiate receptor (Davies & Dray, 1977).

This study was undertaken to determine the anti-SP activity of compounds known to possess analgesic action, on the spasmogenic response to SP in the guinea-pig isolated ileum. It was envisaged that it might be possible to explain the analgesic activity of certain compounds on the basis of SP antagonist activity. The guinea-pig ileum preparation was subsequently used in an attempt to identify a specific antagonist to SP. The results of a recent study by Bury & Mashford (1977) suggest that SP acts directly on the smooth muscle of the guinea-pig ileum, possibly via a SP receptor. In addition, a prejunctional action of SP on cholinergic transmission has been suggested by Hedqvist & von Euler (1975).

* Present address: M.R.C. Clinical Pharmacology Unit, Radcliffe, Infirmary, Oxford, U.K.
† Correspondence.

Segments from the caecal end of the guinea-pig ileum were suspended in a 10 ml organ bath containing Krebs-bicarbonate solution gassed with 5% CO_2 in oxygen. Synthetic substance P (Beckman Inc. or Dr J. S. Morley, ICI Alderley Park) was added at 3 min intervals and left in contact with the tissue for 30 s before washing. Test compounds were made up in Krebs-bicarbonate solution unless otherwise stated and were added to the bath solution 2 min before the addition of SP. Contractions were recorded isotonically. Each compound was tested on a fresh piece of ileum obtained immediately after an animal had been killed. Earlier experiments had demonstrated that if the tissue had been allowed to stand in Krebs solution for longer than an hour before use, then the sensitivity of the tissue to SP was initially higher than that observed in tissue from a freshly killed animal. This increased sensitivity did however, gradually subside after repeated regular dosing with SP. Control dose-response curves to SP were defined both before and after that obtained in the presence of the test compound.

At concentrations up to 10 μM the peripherally acting analgesics indomethacin (MSD), paracetamol (Winthrop), ketoprofen (M & B), naproxen (Syntex and ibuprofen (Boots) failed to antagonize the contractions to SP.

The opiate receptor antagonist naloxone (Winthrop), at concentrations up to 15 μM, also failed to modify the contractile responses to SP.

The opiate receptor agonists morphine, codeine, pethidine (Macfarlan Smith), and diethylthiambutene

(Burroughs Wellcome) were similarly ineffective at concentrations up to 20 μM. Phenoperidine and fentanyl (Janssen) both caused reversible inhibition of the response to SP (Table 1). With phenoperidine the contractions produced by acetylcholine and histamine were more sensitive to blockade by this compound than were those elicited by SP. The antagonism to the response elicited by SP was not reversed by naloxone.

Among a range of non-opiate drugs producing analgesia, and in some instances with unknown modes of action, nefopam (Riker), baclofen (Ciba) and cocaine (Macarthys) were all ineffective, at concentrations up to 30 μM, against the spasmogenic effect of SP.

In acetic acid solution the butyrophenones haloperidol (Searle), pimozide and droperidol (Janssen) all caused irreversible inhibition of the response to SP (Table 1). Acetic acid alone did not affect the response to SP. The commercial preparation of droperidol reversibly antagonized the response to SP but at concentrations 1000 times higher than in acetate solution. A discrepancy between the real and intended concentrations of butyrophenones in aqueous solutions, due to adsorption to glass or plastic, has been noted elsewhere (Seeman, 1977) and may be the cause of the apparent difference in affinities here.

Stern & others (1961) claimed to have found several specific antagonists of SP using the isolated ileum preparation. The SP used in their assays was a bio-extract from animals brains. The two most potent and specific antatonists were trimetaphan camsylate (Roche) and L-cystine-di β-naphthylamide (Sigma). In the present experiments both these compounds inhibited the response to synthetic SP, but with poor specificity (Table 1).

Table 1. *Inhibition of the response to substance P (SP), acetylcholine (ACh) and histamine (Hist) produced by test compounds on the isolated guinea-pig ileum.* [Inhibition is defined by the concentration required to produce a shift in the dose-response curve equivalent to a dose-ratio of 2].

Test compound	SP	ACh	Hist
Opiates			
Phenoperidine	$1{\cdot}43 \times 10^{-5}$	$6{\cdot}49 \times 10^{-6}$	$3{\cdot}89 \times 10^{-6}$
Fentanyl	$8{\cdot}54 \times 10^{-6}$	—	—
Butyrophenones			
Pimozide	$3{\cdot}63 \times 10^{-8}$*	$2{\cdot}07 \times 10^{-7}$	$5{\cdot}35 \times 10^{-8}$*
Haloperidol	$2{\cdot}44 \times 10^{-8}$*	—	—
Droperidol (acid solution)	$3{\cdot}53 \times 10^{-8}$*		
Droperidol (neutral solution)	$2{\cdot}50 \times 10^{-5}$		
Trimetaphan camsylate	$4{\cdot}35 \times 10^{-5}$		$6{\cdot}06 \times 10^{-5}$
L-Cystine-di-β naphtylamide	$3{\cdot}13 \times 10^{-6}$	$3{\cdot}45 \times 10^{-6}$	$2{\cdot}70 \times 10^{-6}$

* Denotes irreversible inhibition.

These results indicate that none of the compounds tested are specific antagonists of the spasmogenic action of SP on the guinea-pig ileum. Our results with morphine, indomethacin and pimozide are in good agreement with those of Bury & Mashford (1977). If SP is the primary afferent neurotransmitter involved in pain perception, then identification of specific SP receptor antagonists may reveal a new class of analgesic compounds as an alternative to the opiates.

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