Table 2. Lowering of brain DOPAC by amphetamine and *p*-chloroamphetamine in control and mazindol-pretreated rats. (±)-Amphetamine sulphate and (±)-*p*-chloroamphetamine hydrochloride were injected i.p. at 15 mg kg^{-1} 15 min after mazindol (15 mg kg^{-1} i.p.) and 1 h before rats were killed.

Treatment	Brain DOPAC, ng g^{-1} Control	Mazindol-pretreated
Saline	104 ± 4	138 ± 11
Amphetamine	62 ± 3* (−40%)	71 ± 7* (−49%)
p-Chloroamphetamine	44 ± 2* (−58%)	71 ± 5* (−49%)

* Significant lowering compared to the corresponding saline group, $P < 0.01$.

spiperone-induced rise in brain DOPAC (Fuller & Snoddy 1978), did not prevent the decrease in brain DOPAC caused by either amphetamine or *p*-chloroamphetamine.

If amphetamine is actively transported into the dopamine neuron via the membrane uptake pump, and if the lowering of DOPAC by amphetamine depends on its active uptake, then uptake inhibitors should prevent the lowering of DOPAC by amphetamine. However, neither mazindol nor methylphenidate, two of the most active dopamine uptake inhibitors known, prevented the lowering of DOPAC by amphetamine. The ability of methylphenidate to block the stereotypy but not the DOPAC-lowering produced by amphetamine would seem most compatible with the idea that amphetamine is not dependent on the uptake pump for entry into the dopamine neuron but that dopamine released non-exocytotically (Arnold et al 1977) by amphetamine is dependent on the membrane pump for transport out of the neuron (as suggested for noradrenaline by Paton, 1973). Methylphenidate, by preventing the transport of released dopamine into the synaptic cleft, prevented the stereotypy caused by amphetamine in reserpinized rats (our observations and those of Ross, 1978). However, neither methylphenidate nor mazindol prevented the lowering of DOPAC by amphetamine, since that may occur through inhibition by amphetamine of monoamine oxidase attack on dopamine within the dopamine neuron (Braestrup 1977; Green & El Hait 1978). As a consequence, the lowering of brain DOPAC by amphetamine was dissociated from increased stimulation of synaptic receptors by dopamine after amphetamine treatment.

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Bradykinin-induced flexor reflex of rat hind-limb for evaluating various analgesic drugs

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Bradykinin injected through a catheter, previously implanted in the right carotid artery of conscious rats, was found by Deffenu et al (1966) to result in dextrorotation of the head, flexion of the right fore-limb and occasionally squeaking. The technique was extended by Blane (1967) and Abe et al (1971) to the evaluation of analgesic drugs. But with these methods, assessment of the suppressive effect of drugs on the bradykinin-induced responses tends to be subjective, and functional impairment of the brain due to ligation of the right carotid artery may induce changes in susceptibility to analgesics. We report an improved method in which the bradykinin-induced flexor reflex of the hind-limb of the conscious rat was recorded and used as a measure of the nociceptive reaction.

Male Sprague-Dawley rats (200–300 g) under light ether anaesthesia, had a polyethylene cannula (0·6 mm o.d.) inserted retrogradely into the left femoral artery so that the tip was in the left common iliac artery just distal to the bifurcation of the abdominal aorta. Solutions injected through the cannula flowed into the contralateral (right) common iliac artery in which a normal blood flow was maintained. Another cannula inserted into the left femoral vein was used for drugs. Immediately after anaesthesia ended, the animal was suspended horizontally in a sleeve of cloth with slits through which the four limbs, tail and cannula were exposed, all but the right hind-limb and tail being

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loosely restrained. The right limb was linked to a kymograph with a thread for recording flexor reflexes.

At least 2 h after the rat had recovered from anaesthesia, 0·2 ml of 0·9% NaCl was injected and if this did not produce any movement of the right hind-limb, 1–4 μg of bradykinin (Protein Research Foundation, Mino, Japan) in 0·2 ml saline was injected within 1 s at intervals of 10 min.

The drugs used were: nalorphine HCl, pentazocine (Pentagin, Sankyo Co.), morphine HCl, indomethacin, aminopyrine, aspirin, methotrimeprazine maleic acid, methamphetamine HCl, physostigmine sulphate, chlorpromazine HCl (Contomin, Yoshitomi Pharmaceutical Industries, Ltd.), mephenesin (Myanol, Chugai Pharmaceutical Co.) and naloxone HCl. Drugs (except indomethacin, aminopyrine and aspirin) were given intravenously in 0·1 ml saline per 100 g. Indomethacin and aspirin suspended in 0·2% carboxymethyl cellulose Na and aminopyrine dissolved in distilled water were given intraperitoneally.

In the present experiments, the right hind-limb was not disturbed for 7 h then 1–4 μg (usually 2 μg) of bradykinin was injected, when flexor reflexes of the limb were reproduced in 153 (88%) of 174 rats tested. These reflexes had latencies of 4–10 s and terminated within 30 s of the injection. Only rats in which reproducible flexor reflexes were elicited in at least four successive control trials were used. When, after administration of a drug, the magnitude of the reflexes following more than two successive bradykinin injections was less than 25% of the smallest control value and recovered to the control level, the effect of the drug was regarded as being inhibitory, that is analgesic. Each rat was given only one dose of a drug. The ED50 values and 95% confidence limits were determined according to Litchfield & Wilcoxon (1949).

The results and approximate clinical analgesic doses are summarized in Table 1.

Nalorphine and pentazocine which act as agonist-antagonists at opiate receptors showed a strong analgesic action in a dose-dependent manner. As shown in Fig. 1, nalorphine, 0·5 mg kg^{-1}, i.v., immediately suppressed the bradykinin-induced reflex and this was antagonized by naloxone (0·1 mg kg^{-1}, i.v.) given 50 min after nalorphine, while the inhibition lasted over 70 min without naloxone. Pentazocine was also antagonized by naloxone (0·1 mg kg^{-1}, i.v.). The relative potencies of nalorphine and pentazocine correspond roughly to those in clinical use. Morphine's inhibitory effect (Table 1) was antagonized by naloxone (0·1 mg kg^{-1}, i.v.) but its ED50 suggested that this test is less sensitive to morphine than to agonist-antagonists.

Dose-dependent analgesic actions of the anti-inflammatory analgesics were clearly detectable (Table 1). The ED50 values of indomethacin, aminopyrine and aspirin had a potency ranking similar to that seen in clinical use.

Table 1. Analgesic activity of various drugs evaluated by inhibition of bradykinin-induced flexor reflex of rat hind-limb and approximate analgesic doses in man (N.E. = no effect).

	ED50 mg kg^{-1} (95% confidence limits)		Approx. clinical analgesic doses (mg)	
Nalorphine	0·17 (0·09–0·32)	i.v.	10–15	s.c./i.m.
Pentazocine	1·00 (0·63–1·58)	i.v.	30–50	s.c./i.m.
Morphine	1·45 (1·07–1·96)	i.v.	10–15	s.c./i.m.
Indomethacin	38·0 (16·5–87·4)	i.p.	25–50	oral
Aminopyrine	41·0 (20·5–82·0)	i.p.	100	oral
Aspirin	100 (43·5–230)	i.p.	300–600	oral
Methotrimeprazine	N.E. 3·0–10	i.v.	15–30	i.m.
Methamphetamine	N.E. 0·5–2·0	i.v.	—	
Physostigmine	N.E. 0·01–0·05	i.v.	—	
Chlorpromazine	N.E. 3·0 –10	i.v.	—	
Mephenesin	N.E. 30–70	i.v.	—	

Methotrimeprazine, which has about half the potency of morphine (Montilla et al 1963), was not effective at 3–10 mg kg^{-1}, i.v. On the contrary, Kuromi et al (1972) reported that 1 mg kg^{-1}, i.v. of the drug blocked the trigeminal pain evoked by electrical stimulation of the tooth pulp of the rabbit. These findings suggest that methotrimeprazine may influence a specific but not general nociceptive mechanism.

Methamphetamine, 0·5–2·0 mg kg^{-1}, i.v., produced no depressant action on the bradykinin response but increased spontaneous movements of the animals.

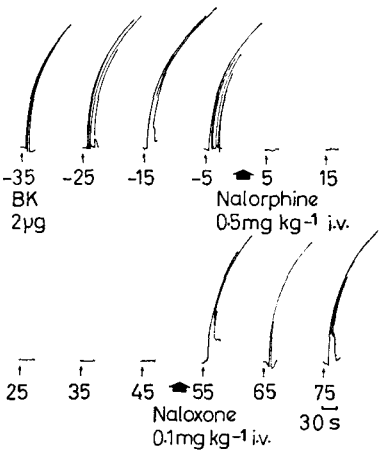

FIG. 1. An example of inhibitory action of nalorphine (0·5 mg kg^{-1}, i.v.) on the flexor reflexes induced by bradykinin injected into the common iliac artery and antagonism to the inhibition by naloxone (0·1 mg kg^{-1}, i.v.). Minus signs indicate time before drug administration.

Moderate to high doses of non-analgesic drugs such as physostigmine (0·01–0·05 mg kg^{-1}, i.v.) chlorpromazine (3–10 mg kg^{-1}, i.v.) and mephenesin (30–70 mg kg^{-1}, i.v.) did not produce any inhibitory effect, although they are effective in the mouse writhing test (Charnov et al 1967). This indicates that the present method is probably the more specific of the two for the evaluation of analgesics.

Our present findings suggest that the method described overcomes the drawbacks in the anti-bradykinin test previously reported by Blane (1967) and Abe et al (1971) and is useful for testing various analgesics, particularly narcotic agonist-antagonists and anti-inflammatory analgesics.

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The inhibitory effects of sex steroid hormones on electrically-induced contractions of the guinea-pig isolated ileum

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Progesterone, pregnenolone, testosterone, ethinyloestradiol, oestrone and oestriol reversibly inhibit guinea-pig isolated ileum contractions to PGE_1 and $F_{2\alpha}$ (Seaman et al 1978), acetylcholine, histamine (Seaman et al 1977a) nicotine and 5-hydroxytryptamine (5-HT) (Seaman et al 1977b) and these four last inhibitions can be reversed by PGE_1 or $F_{2\alpha}$. It was concluded that sex steroids exert a spasmolytic effect on ileal smooth muscle which was also the conclusion of Ishida et al (1972) who noted a papaverine-like action of some sex hormones on the guinea-pig ileum. These inhibitory actions are similar to those induced by non-steroidal and steroidal anti-inflammatory drugs (NSAID and SAID) (Famaey et al 1975, 1977a,b). These anti-inflammatory compounds are also able to inhibit guinea-pig isolated ileum contractions induced by transmural electrical stimulations and these inhibitions are reversed by PGE_1, E_2 and $F_{2\alpha}$ (Famaey et al 1975, 1977a), caerulein (Fontaine 1976) and metoclopramide (Fontaine et al 1979).

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We have now investigated whether similar inhibitions and similar reversals occur with steroid sex hormones.

Four cm lengths of guinea-pig ileum were suspended under an initial load of 1 g in Krebs-Henseleit solution

Table 1. Inhibitory effects of two sex steroid hormones on the responses of the guinea-pig isolated ileum to coaxial stimulation (12 min contact time). The results (mean ± s.e.m., n = 6) have been analysed with the Student's *t*-test for paired data.

Sex steroids	μg ml^{-1}	% inhibition
Testosterone	0·5	47* ±9
	1	62*** ±10
	5	96*** ±2
Ethinyloestradiol	0·5	21* ±5
	1	53** ±11
	5	96*** ±2

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$