The effects of noradrenaline and 5-hydroxytryptamine on the responses of dorsal horn neurones to noxious and innocuous skin stimuli

A.W. DUGGAN & P.M. HEADLEY

Department of Pharmacology, John Curtin School of Medical Research, Australian National University, P.O. Box 334, Canberra City, ACT, 2601, Australia

There are numerous reports relating noradrenergic (NA) and tryptaminergic (5-HT) systems to the analgesia induced both by morphine and by stimulation in the midbrain (see Oliveras, Hosobuchi, Redjemi, Guilbaud & Besson, 1977, for references). Such an interaction could occur at the termination of descending NA or 5-HT fibres in the substantia gelatinosa of the spinal cord, a region in which morphine and methionine enkephalin amide have been shown to be active in reducing the nociceptive responses of neurones in laminae IV and V (Duggan, Hall & Headley, 1976, 1977). We have now tested NA and 5-HT administered electrophoretically in the dorsal horn.

Experiments were performed on 12 spinal cats anaesthetized with α-chloralose, paralysed with gallamine and artificially ventilated. The noxious stimulus to a hindlimb footpad was radiant heat (skin temperature $>45°C$). Such a stimulus was alternated with an innocuous stimulus produced by deflection of nearby hairs by a moving airjet. Extracellular recordings were obtained with a multibarrel pipette positioned in spinal lamina IV or V, and drugs were ejected either from this pipette or from a second electrode positioned in the substantia gelatinosa, 50–760 μm dorsal and up to 100 μm lateral to the recording electrode. Results were obtained from 26 cells.

Administered in the substantia gelatinosa, NA (20–100 nA) and 5-HT (30–250 nA) reduced the nociceptive responses of 20/20 and 13/19 cells respectively. Selectivity (i.e. no reduction of responses to innocuous stimuli) occurred with 9 cells using NA and 6 cells using 5-HT; with most other neurones non-nociceptive responses were also reduced but to a lesser extent than were nociceptive responses. The time course of these effects was prolonged. Recovery from NA took 2–15 min and with 5-HT recovery took more than 20 min on 7 of 13 occasions. These actions contrast with the lack of effect of acetylcholine (150–200 nA, 7 cells), GABA (100–200 nA, 11 cells) and excitant amino acids (Duggan *et al.*, 1977).

Ejected near cell bodies 5-HT (22–100 nA) reduced both nociceptive and non-nociceptive responses of 4 of 6 cells tested and GABA (12–100 nA) was similarly non-selective on 7 of 10 cells. NA (10–40 nA) was slightly selective on 9 of 10 cells, but the degree of this selectivity was much less than with administration into the substantia gelatinosa.

Thus the monoamines, but not amino acids, were similar to morphine and enkephalin in being more selective for nociceptive responses when administered in the substantia gelatinosa. The opiate antagonist naloxone reduces the analgesia produced by stimulation in a nucleus of origin of descending 5-HT fibres (Oliveras *et al.*, 1977); we are currently testing naloxone for interaction with the monoamines administered in the substantia gelatinosa.

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Correlation between effects of brain-stem stimulation and effects of 5-hydroxytryptamine and noradrenaline on non-nociceptive and nociceptive spinal interneurones

G. BELCHER (introduced by R.W. RYALL)

Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD

Electrical stimulation in the area of the brain stem raphé nuclei produces analgesia in the cat (Mayer & Liebeskind, 1974) which is thought to be produced by activation of a descending influence to cause inhibition of nociceptive impulses at the spinal level (Liebeskind, Guilbaud, Besson & Oliveras, 1973). It has been suggested that this descending pathway is tryptaminergic (Guilbaud, Besson, Oliveras & Liebeskind, 1973; Vogt, 1974). In order to investigate this possibility further, 5-hydroxytryptamine (5-HT) and noradrenaline (NA) were applied locally to identified dorsal horn interneurones and an attempt to correlate their effects on spontaneous and stimulus-evoked firing with effects of brain stem stimulation on their activity was made.

Cells were identified as having a nociceptive input if their activity was altered by injection of small amounts

of bradykinin into the blood supply of their peripheral receptive field. Electrical stimulation in the area of the central interior mucleus of raphé generally caused inhibition of nociceptive cells but about half the cells classified as non-nociceptive were also inhibited to a lesser degree and for a shorter duration. On many cells, even when inhibition was seen, brain stimulation evoked one or two action potentials at short latency, prior to the inhibition, and there was no correlation with nociceptive input.

The effect of 5-HT on spontaneous activity was excitatory on both nociceptive and non-nociceptive neurones but it selectively inhibited activity evoked by noxious stimuli on nociceptive cells, indicating a pre-synaptic site of action. In contrast, NA generally had little effect on non-nociceptive cells whilst causing long-lasting inhibition of nociceptive cells and there was a correlation between the amount of inhibition (measured as the percentage inhibition of control firing) caused by NA and the percentage inhibition from brain stem stimulation.

From these results it would appear that 5-HT is unlikely to be a candidate as the transmitter mediating inhibition from brain stem stimulation. However, descending excitatory tryptaminergic pathways may increase activity of interneurones which in turn inhibit other neurones transmitting nociceptive impulses.

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On the structural requirements for dopamine-like activity in homogenates of rat nucleus accumbens

K.A. MUNDAY, JUDITH A. POAT, K.J. WATLING & G.N. WOODRUFF

Department of Physiology & Biochemistry, University of Southampton

The dopamine-sensitive adenylate cyclase present in homgenates of rat striatum (Kebabian, Petzhold & Greengard, 1972) has proved to be a useful model system for the study of drugs affecting mammalian dopamine receptors (review by Iversen, 1975). Dopamine sensitive adenylate cyclases are also present in other brain regions including the nucleus accumbens (Horn, Cuello & Miller, 1974). We have now investigated the structural requirements for dopamine-like activity on the adenylate cyclase from rat nucleus accumbens.

The activity of adenylate cyclase in homogenates of rat nucleus accumbens was estimated using the method of Kebabian, Petzhold & Greengard (1972) and the resulting cyclic AMP was determined according to Gilman (1970). Potency of agonists was expressed as a percentage of the maximum response which was taken as that produced by 100 μM dopamine. A log concentration/response curve was constructed for each compound and EC_{50} values (concentration producing 50% of maximum response) were obtained.

In the absence of dopamine, the mean production of cyclic AMP by 50 μl nucleus accumbens homogenates was 49.9 ± 9.4 ($n = 12$) pmol/tube during the 3 min incubation. This was increased to 80.2 ± 9.4 ($n = 12$) pmol/tube by 100 μM dopamine. The potency of the compounds tested is shown in Table 1.

Table 1 Potency of drugs in increasing cyclic AMP production in homogenates of rat nucleus accumbens

Agonist	EC_{50} (μM)
ADTN*	0.7 ± 0.3
Epinine	1.8 ± 0.8
Dopamine	6.3 ± 1.2
(−)-Noradrenaline	25.1 ± 14.6
(−)-Adrenaline	50.1 ± 17.2
3,4-dihydroxy-5-methoxy phenylethylamine	141.3 ± 17.2
(±)-Isoprenaline	inactive
(−)-Phenylephrine	inactive
3,5-dihydroxy-4-methoxy phenylethylamine	inactive
3-hydroxy-4,5-dimethoxy phenylethylamine	inactive

* 2-Amino-6,7-dihydroxy-1,2,3,4-tetrahydro-naphthalene.
EC_{50} values are the mean ± s.e. mean obtained from 4–8 observations.
All active compounds gave 100% stimulation.