Multiple brain sites involved in morphine antinociception

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Stimulation of peripherally located pain receptors by different techniques leads to a nociceptive reaction which can be strongly delayed by systemic morphine treatment. A similar effect can be obtained when morphine is injected directly into the brain ventricular system or into various brain structures (Herz, Albus & others, 1970; Pert & Yaksh, 1974; Yaksh, Yeung & Rudy, 1976). I report an attempt to localize the antinociceptive effect of morphine in more detail.

The nociceptive response of Wistar rats, 120–150 g, was determined using a hot plate device (54·2 ± 0·1°) according to Eddy & Leimbach (1953). The criterion of the response was the licking of one of the paws or intensive jerking with lifting off or jumping on the hind legs. Cut-off time in the absence of a response was 60 s. Morphine hydrochloride (morphine) solutions were prepared in physiological saline. All doses refer to the salt.

Two sets of experiments were performed. First, various brain sites were mapped for antinociceptive activity by unilateral injections of morphine. The preparation of the 60 female animals used for the injection has been described previously (Hulst & de Wied, 1967). Briefly, a stainless steel plate with 12 holes and tubes was fixed to the skull. Through the tubes needles of different length could be directed into the brain. After a recovery period of at least one week, the rats were injected on three consecutive test days with graded doses of morphine: 2, 3 and 4 μg per 2 μl (pH of solution 7·35). The injection sites in every animal were randomly placed throughout the brain. Response latency of the animals was determined before and 30 min after morphine injection. Following completion of the experiment, the rats were killed and the brains prepared following standard histological procedures. The micro-injection sites were located by microscopic examination and the positions determined according to the atlas of Pellegrino & Cushman (1967).

In a second set of experiments, male rats were equipped with a polyethylene cannula in one of the brain ventricles. The coordinates of the tip of the cannula for the lateral ventricle were 1 mm lateral to the midline, 0·5 mm caudal to the bregma, and 4·5 mm from the surface of the dura; those for the 4th ventricle: the midline, 2 mm caudal to the lambda and 6·8 mm from the surface of the dura; those for the 3rd ventricle, the midline, 1 mm caudal to the bregma and 7·5 mm from the surface of the dura. Groups of animals received various doses of morphine injected into one of the ventricles and response latency was determined before and 20, 40 and 60 min after injection. Injection volume was always 1 μl. Each animal received one injection only. The localization of the tip of the cannula was determined at the termination of the experiment by the injection of azovan (Evans) blue. The staining was then inspected macroscopically in formaldehyde fixed brain sections.

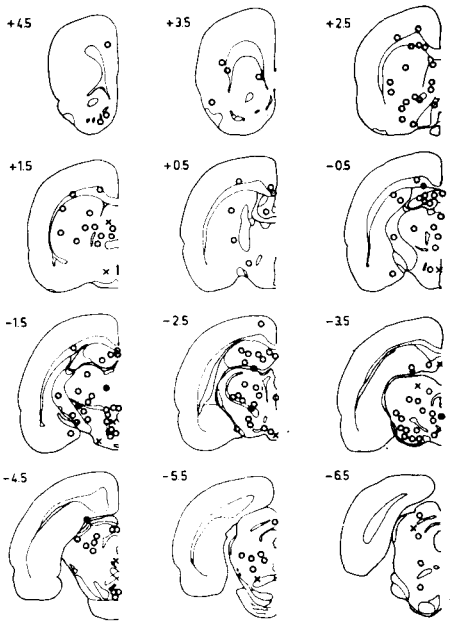

FIG. 1. Anatomical mapping of the antinociceptive sites of action of morphine in the rat brain. The graphs refer to planes according to the atlas of Pellegrino & Cushman (1967) with the bregma as reference point (mm rostral (+) or caudal (−) to the bregma). Sites are shown where microinjection of morphine (2–4 μg) elicited a marked effect (●, increase in response latency >40 s), a moderate effect (×, increase in response latency between 25 and 40 s) or no effect (○, increase in response latency <25 s).

The effect of morphine micro-injected into 164 brain sites is presented in Fig. 1. A marked antinociceptive effect was found when morphine was injected into or near the ventricles, the substantia grisea centralis, the lemniscus medialis and the nucleus mediodorsalis thalami. Moderate antinociceptive activity was observed after injections into sites of the formation reticularis, into the nucleus lateralis septi, the nucleus anterior and posterior hypothalami, the nucleus mamillaris lateralis and the colliculus superior and inferior. Placebo injections into various brain sites did not change the response latency of the rats.

Table 1 shows the response latency of animals before and 40 min after intraventricular injection of various doses of morphine. Response latencies obtained at

Table 1. *Nociceptive response of rats as assessed on the hot plate before and 40 min after various doses of morphine HCl injected into different parts of the brain ventricle system.*

Dose of morphine (μg)	Site of inj.*	Time: Before treatment (s ± s.e.m.)	Time: After treatment (s ± s.e.m.)	n
0·625	1	9·1 ± 0·6	12·8 ± 2·8	9
	3	8·5 ± 1·4	9·9 ± 1·6	7
	4	8·8 ± 0·7	21·2 ± 5·4	10
1·25	1	8·1 ± 0·6	17·3 ± 4·9	8
	3	9·5 ± 0·7	17·4 ± 5·5	9
	4	8·2 ± 0·5	30·4 ± 6·1	8
2·5	1	9·0 ± 0·6	20·6 ± 5·5	10
	3	9·1 ± 0·7	21·5 ± 6·7	10
	4	9·7 ± 0·6	33·7 ± 6·8	10
5·0	1	9·9 ± 0·7	44·3 ± 6·4	10
	3	8·6 ± 0·7	42·0 ± 7·3	9
	4	10·3 ± 0·9	46·0 ± 6·3	8
20	1	9·6 ± 1·0	52·0 ± 6·2	9
	3	10·5 ± 1·3	52·1 ± 5·8	8
	4	9·6 ± 0·9	49·2 ± 6·4	9

* 1 = lateral ventricle; 3 = 3rd ventricle; 4 = 4th ventricle.

20 and 60 min after injection were not substantially different. In general, peak activity was observed at 40 min after morphine injection. Morphine, 0·625 μg, injected into the 4th ventricle elicited antinociceptive activity. Four times this dose was needed to obtain a similar response when the drug was applied in the lateral or the 3rd ventricle. Analysis of variance of the data obtained with the three lowest doses indicated a significant difference between the groups with respect to the site of injection ($P = 0{\cdot}01$). Further analysis indicated that injections in the 4th ventricle were more effective than those in the lateral or 3rd ventricle (respectively $P < 0{\cdot}05$ and $< 0{\cdot}05$). Interestingly, increasing the dose of morphine from 2·5 to 5 μg resulted in a marked increase in effectiveness especially when the drug was injected into the lateral or the 3rd ventricle.

Studies dealing with the neuroanatomy of the pain pathways and associated structures, indicate the existence of two anatomically and functionally distinct systems: the extralemniscal somatosensory system mediating the affective-motivational component of pain and the neospinothalamic tract mediating the sensory-discriminative aspect of pain (Casey & Melzack, 1967; Albe-Fessard, 1968). The distribution of active brain sites as found in the present experiments is roughly similar to that observed in rabbits and monkeys (Herz & others, 1970; Pert & Yaksh, 1974) and suggests that most of the active sites are more or less related to the extralemniscal somatosensory pathways. This paramedial ascending system transmits information from the spinal cord to the reticular formation, the limbic midbrain area and the medial thalamus and has a strategic relation to the limbic system and associated structures which have been found to play a profound role in motivational processes (Casey & Melzack, 1967). As has been suggested this extralemniscus system mediates the affective-motivational component of pain. Thus, it might be postulated that morphine primarily affects the motivational value of pain stimulation. However, it should be borne in mind that diffusion of morphine into the ventricular system and subsequent transport to other brain structures may have contributed to the morphine-induced analgesia in the present experiment. Furthermore, an effect of morphine—presumably at higher dose level—on the lemniscus tract which mediates the sensory-discriminative aspect of pain, cannot be excluded, since some active sites were found close to the lemniscus medialis (see also Pert & Yaksh, 1974).

Morphine injections into the 4th ventricle were more effective in producing analgesia than injections into other parts of the ventricular system. This suggests that structures around the 4th ventricle are highly sensitive to morphine, or that structures belonging to the pain pathways, in particular those related to the extralemniscus system, are located closer to the ventricular system in the lower brain stem. This agrees with the conclusion of Yaksh & others (1976) that in the rat the periaquaductal gray is one of the most sensitive supraspinal sites with respect to morphine-induced antinociception.

Although analgesia was already observed after injection of 625 ng of morphine into the 4th ventricle, it must be stressed that this is still a relatively high dose. Preliminary results indicate that 30 min after an intraveneous injection of 2·25 mg kg^{-1}, morphine concentrations in the subcortex appear to be about 60 ng g^{-1} tissue (Plomp, Maes & van Ree, unpublished data). Thus, systemically injected morphine might affect central pain pathways at different levels, which in some way or other might contribute to the observed analgesia. The existence of multiple sites of morphine action is supported by the observations that morphine sensitive sites are also present at the ventral surface of the brain stem and in the spinal cord (Rosenfeld & Kowatch, 1975; Dey & Feldberg, 1976; Yaksh & Rudy, 1976). It also explains the marked increase in antinociceptive response when the amount of intraventricularly injected morphine was increased from 2·5 to 5 μg.

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Bupropion hydrochloride ((±) α-t-butylamino-3-chloropropiophenone HCl): a novel antidepressant agent

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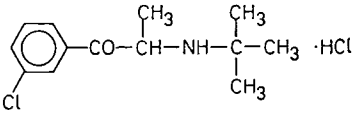
Tricyclic compounds such as amitriptyline and related substances are considered to be clinically effective antidepressant agents (Kuhn, 1958; Hollister, 1972) with a mode of action dependent on the inhibition of the neuronal reuptake of one or more biogenic amine transmitters in the central nervous system (Glowinski & Axelrod, 1964). The tricyclics have not proved to be ideal agents in treating depression due to their slow onset of action, cholinolytic side effects, interactions with pressor amines and monoamine oxidase inhibitors and also due to a tendency to elicit cardiac arrhythmias or standstill (Jefferson, 1975). Monoamine oxidase inhibitors are likewise considered to be effective antidepressant agents (American Psychiatric Association, 1974) which act by inhibiting intraneuronal monoamine oxidase in the central nervous system (cns). However, the currently available inhibitors also inhibit monoamine oxidase in the liver and the ingestion of phenethylamine-type pressor substances in the diet, normally an event made innocuous with the effective destruction of the amines by liver monoamine oxidase, may lead to hypertensive crisis (Marley & Blackwell, 1970).

We therefore sought an agent that would be active in antidepressant screening models, but differ chemically and pharmacologically from the tricyclics, and not be sympathomimetic, cholinolytic nor an inhibitor of monoamine oxidase. Bupropion (Wellbatrin) which was synthesized by one of the authors (N.B.M.) (Baltzly & Mehta, 1968; Mehta, 1974, 1975) meets these criteria and has the following structure:



The pharmacological properties of bupropion have to date been reported only in abstract form (Soroko, Mehta & others, 1970) as has its clinical effectiveness as an antidepressant in an open study by Fann, Schroeder & others (1974) and in a double-blind, placebo-controlled study by Fabre, McLendon &

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