A COMPARISON OF THE SPASMOLYTIC EFFECTS OF TWO PHENYLETHYLAMINES AND SOME OBSERVATIONS ON MORPHINE-LIKE ACTIVITY

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Contraction of isolated guinea pig ileum caused by 5-hydroxytryptamine, acetylcholine, histamine and barium was prevented by the isopropyl and cyclohexyl ethers of 1-(*p*-hydroxyphenyl)ethylamine at similar concentrations to those found in rat tissues after injection. Inhibition due to the isopropyl ether was immediate and readily washed out. It did not antagonise substance P. Inhibition by the cyclohexyl ether was persistent and needed a short incubation period for full potency to develop. It antagonised substance P. The results are introduced against a background of a broad speculation on the nature of morphine-like activity. Ammonium ion partially inhibited 5-hydroxytryptamine at a concentration similar to that found *in vivo* during convulsions.

THE sum total of morphine's effects in the animal seems to be a good example of Gunn's pharmacological syndrome[1]. It could be a physiological syndrome in the manner that the adaptation syndrome or sympathomimesis can be so regarded. It is not difficult to portray the morphine syndrome as an antithesis and subsidiary to sympathomimesis. It is such as might be expected of a non-specific summation of changes that occur after receipt of a variety of noxious stimuli, changes that severally can assist the maintenance of passive states. These are characterised in the main by immobility whereas sympathomimesis fits the animal for the mobility of fight or flight. Well defined passive states include hibernation, feigned death and the immobility of "freezing". They are initiated by noxious stimuli that for reasons such as a lack of orientation in space or insuperability, are difficult to avoid. When fight or flight is impracticable, protection may often be conferred by immobility. This speculative and ramifying topic cannot be elaborated here but two points need emphasis. No drug randomly distributed by blood can be expected to mimic faithfully the various protective reactions that arise after permeation of sensory information through the filter of nervous integration. At best there can be a non-specific and qualitative summation of various reactions. Morphine need not induce a passive state, just as adrenaline need not cause fight or flight.

On the above grounds and by analogy with sympathomimesis, it was surmised that morphine and its substitutes could either imitate, release, inhibit or modify tissue response to, some substance concerned with the maintenance of state and, by hypothesis, associated with a diffuse efferent neural network. So far, only adrenaline, and possibly cortisol, have been recognised as natural agents that help to maintain a change in state in the body as a whole, though local hormones[2] appear to control the state, such

as tone, of individual organs. "Inhibitory transmittors" have been postulated from time to time to explain experimental findings but it was convenient in this instance to consider some known humoural substances first. Of these, 5-hydroxytryptamine and substance P have most interest relative to morphine. The richest tissue sources of both are the autonomic brain and intestine, tissues that are probably directly influenced by the drug.

The effects of substance P in the whole animal tend to run counter to those of morphine. It rendered mice hyperalgesic and antagonised morphine analgesia[3]. The animals were sedated[3,4]. It induced tachypnoea and hyperpnoea[5]. Conversely, like morphine, it was a transient depressor agent by vasodilation and increased intestinal tone[6]. The distribution in tissues has suggested an association with the first sensory neurone[7]. 5-Hydroxytryptamine was a potent pain producing agent[8]. Morphine antagonised 5-hydroxytryptamine spasm in guinea pig ileum[9,10] and caused a lasting depression of the indole's effect on the superior cervical ganglion[11]. Morphine released the indole from rat tissues[12]. The depressant effect of 5-hydroxytryptamine on brain was antagonised by morphine[13], whereas the psychomotor effects of morphine were antagonised by reserpine[14,15].

A simple experimental approach was to compare the spasmolytic effect of the isopropyl and cyclohexyl ethers of 1-(*p*-hydroxyphenyl)-ethylamine on the isolated intestine. This tissue may provide a model of certain hypothalamic processes[16] with the advantage that the end result of any action is observable as a muscle movement rather than a synaptic discharge. Agents that influence intestinal activity often influence analgesia. Papaverine may potentiate morphine analgesia[17]. Antihistamines[18,19] have been described as analgesic. Anticholinergic compounds potentiated analgesia[20] and morphine reduced acetylcholine output by isolated intestine[16,21]. There are numerous descriptions of the analgesic effect of adrenaline[22].

During several years the above-mentioned amino ethers have been shown to behave almost identically in a variety of experimental situations but only the cyclohexyl ether mimicked morphine in the rat[23]. The mimicry extended to antagonism of analgesia by the *N*-allyl ether[24]. It seemed that any notable difference between the two ethers could indicate an approach to the mechanism of morphine-likeness. Notable differences were seen in the influence of high concentrations (4×10^{-2}M) on the intestine such as occurs by intraperitoneal injection. The cyclohexyl ether abolished intestinal movement in rats and caused a petechial haemorrhage with a serous exudate containing cholesterol and protein. After several daily doses there was a flaccid distension of the intestine. Morphine in single doses enhances the tone of the muscle but distension has been described in morphine addicts[25]. These effects were never seen in many experiments with the isopropyl ether. As for morphine, some of the effects seem reminiscent of histamine release but, using the Evans blue permeability test, no difference could be found in the ability of the two amines to release histamine from rat skin.

This paper is concerned with a comparison of the spasmolytic effect of the two ethers against the common spasmogenic agents used in investigating drug effects on the intestine.

Methods and Materials

Substance P was extracted either from fresh horse intestine (method of Pernow[26] to the alcohol precipitation stage) or was obtained in solution from the pooled intestines of six rats by a similar method. 2·5 kg. of horse tissue gave 2·04 g. of a buff powder containing 30 per cent ammonium sulphate. It caused contraction of guinea pig ileum at 4 to 20 μg./ml. but appeared to contain 5-hydroxytryptamine since the contraction was partially antagonised by tryptamine. Dialysis and short alkaline but not acid hydrolysis, destroyed the activity but tryptic digestion was only partially effective. The rat preparation contained no 5-hydroxytryptamine and was active at 2 μl./ml. For assay, a bath of 5 ml. capacity was used at 37°. When substance P and 5-hydroxytryptamine were the spasmogenic agents the bathing Tyrode contained atropine and mepyramine (10^{-6})[26].

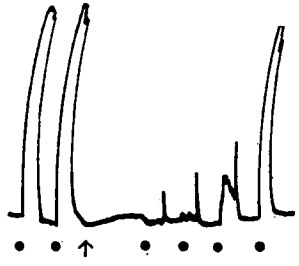

FIG. 1. Inhibition of contractile effect of substance P on guinea pig ileum by 1-(*p*-cyclohexyloxyphenyl)-ethylamine. 20 μl. of substance P solution prepared from rat intestine added at ●. 2×10^{-5}M amine added at the arrow, followed by 3 min. incubation.

Substance P was assayed in tissues after injection of drugs into rats. The tissue was extracted with hot hydrochloric acid at pH 4 for 2 minutes. Substance P was precipitated by saturation with ammonium sulphate and the precipitate extracted with 90 per cent ethanol. The extract was dried at 0°/5 mm. and the residue preserved at −10° until required. About 200 mg. of ammonium sulphate contaminated the extract, sufficient to give a concentration of about 10^{-5}M in the bath. Recovery of known amounts of substance P activity averaged 55 ± 6 per cent.

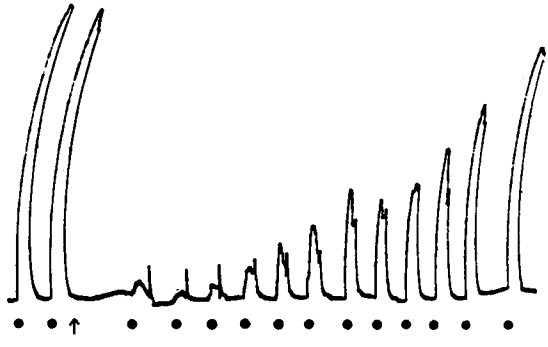

FIG. 2. Prolonged inhibition of contractile effect of 5-hydroxytryptamine on guinea pig ileum by 1-(*p*-cyclohexyloxyphenyl)-ethylamine. 5 μg. 5-hydroxytryptamine creatinine sulphate added at ●. 2×10^{-5}M amine added at the arrow, followed by 3 min. incubation.

RESULTS

Attempts to reduce the substance P content of rat intestine and brain by drugs were unsuccessful. Morphine (10 mg./kg. 30 minutes before killing), reserpine (3 mg./kg. 24 hours before killing) and thyroxine (6 daily doses of 50 μg.) all intraperitoneally, were ineffective.

Both the isopropyl and cyclohexyl ethers of 1-(*p*-hydroxyphenyl)-ethylamine inhibited the contraction of guinea pig ileum caused by acetylcholine (0·1 μg.), histamine phosphate (1 μg.), 5-hydroxytryptamine (2 μg.) and barium chloride (0·2 mg.). The amounts quoted refer to a particular experiment where the doses were adjusted to give equal heights of contraction in the one piece of tissue. There was little to distinguish the immediate inhibitory effect of 2×10^{-5}M concentration of either ether. Inhibition by the isopropyl ether was removed by a single wash and it did not antagonise substance P. Inhibition by the cyclohexyl ether was not always seen immediately after addition but, allowing 3 minutes' contact with the tissue, followed by a single washing, there developed a strong antagonism of the above agents. Substance P was also antagonised (Fig. 1). Removal of the inhibition needed up to twelve washes (Fig. 2). By allowing the inhibition to develop, an effect could be shown against substance P at 2×10^{-6}M. Morphine did not inhibit substance P. A range of phenylethylamines, including ephedrine, hordenine, amphetamine, adrenaline, noradrenaline, mescaline and tryptamine, and a variety of aliphatic amines, all failed to antagonise substance P. It was notable that ammonium ion could partially antagonise 5-hydroxytryptamine at $1–2 \times 10^{-3}$M (Fig. 3). Concentrations of this order (0·6 μM/g.) have been found in brain during convulsions[27].

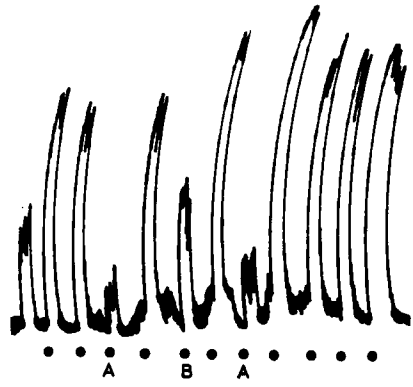

FIG. 3. Inhibition of contractile effect of 5-hydroxytryptamine on guinea pig ileum by ammonium ion. 4 μg. 5-hydroxytryptamine creatinine sulphate added at ●. 2×10^{-3}M ammonium ion added at A. 10^{-3}M ammonium ion added at B.

DISCUSSION

The spasmolytic effects of the isopropyl and cyclohexyl ethers of 1-(*p*-hydroxyphenyl)ethylamine, while non-specific and virtually alike in immediate potency, appear to differ insofar as the cyclohexyl ether needed a period of incubation for its full activity to develop. The inhibition was then persistent. Its ability to inhibit substance P at concentrations as low as 2×10^{-6}M constitutes a distinct difference from the isopropyl ether and may contribute to its effects in the whole animal since concentrations up to 0·3 μmoles/g. (3×10^{-4}M) have been found in rat brain after analgesic doses[28]. Inhibitors of substance P have not been described and

the result suggests that its morphine-like character may arise from this property. Zetler has recently speculated on the role of substance P in morphine analgesia. If the supposition is correct, the mode of analgesic action must differ in detail from that of morphine, which fails to antagonise substance P. It seems possible that a solution to the problem of morphine-like activity could be found in neurohumoural interplay, especially regarding 5-hydroxytryptamine and substance P. Thus it is of interest that the action of morphine on the intestine declines from the duodenum towards the colon and so also does the substance P content[29]. It seems reasonable that the quiescence of the intestine after intraperitoneal injection of the cyclohexyl ether is a consequence of the persistent antagonism to substance P and 5-hydroxytryptamine.

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After Dr. McCoubrey presented the paper there was a DISCUSSION.