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Analysis of the role of withdrawal state in morphine-induced supersensitivity to acetylcholine in mouse ileum

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An attempt has been made to study the possible role played by the withdrawal state in morphine-induced supersensitivity to acetylcholine in mouse isolated ileum. Chronic morphine treatment for 5 days produced a significant supersensitivity to acetylcholine in the ileum. This supersensitivity was not altered significantly by the addition of morphine to the bathing medium—a measure to avoid the precipitation of the withdrawal state. This indicates that the supersensitivity obtained does not represent the state of withdrawal and is the result of chronic morphine treatment.

Collier (1968) explained the tolerance and dependence on opiates on the basis of their inhibitory effect on neurotransmitter release and the subsequent development of supersensitivity in the postsynaptic site. Since then, attempts have been made to associate the development of tolerance and dependence with the sensitivity changes in the post-synaptic site. Certain earlier experiments revealed no such association (Haycock & Rees 1972; Goldstein & Schulz 1973) while recent reports have clearly demonstrated that the development of tolerance to and dependence on opiates significantly correlated with the supersensitivity to neurotransmitters induced by morphine in smooth muscles (Rae et al 1977; Johnson et al 1978; Contreras & Marti 1979; Ramaswamy et al 1980, 1981). North & Vitek (1980) attributed the noradrenaline (NA) supersensitivity observed by Rae et al (1977) in mouse vas deferens after chronic morphine treatment, to the withdrawal state since the tissues after dissection were incubated in opiate-free Ringer solution. In reply to this, Rae & De Moraes (1983) have fully analysed this phenomenon and confirmed that the supersensitivity to NA recorded by them was only the result of chronic morphine exposure and does not represent the withdrawal state. We now communicate the results observed in our study on similar lines in mouse ileum.

While trying to analyse Collier's theory, we noticed that tolerance to the analgesic action developed simultaneously with the supersensitivity to acetylcholine (ACh) in mouse ileum, when animals were exposed to chronic morphine treatment (Ramaswamy et al 1981). In this particular experimental design, we chose to rule out the possible role of withdrawal in morphine-induced supersensitivity to ACh in ileum as suggested by North & Vitek (1980).

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Method

After 5 days of morphine sulphate treatment (10 to 40 mg kg^{-1} twice daily s.c.) mice were killed 5 h after the last morphine injection. The ileal tissue was isolated and incubated in either morphine-free Ringer or Ringer containing 300 nM morphine. The composition of the Ringer solution was mM: NaCl 154, $CaCl_2$ 2·4, KCl 5·4, $NaHCO_3$ 6 dextrose 11. After initial equilibration under 0·2 g load, concentration effect curves were obtained isotonically for ACh and the concentration that produced 50% of the maximal response (ED50) was calculated graphically.

Results

Morphine (300 nM) alone, when added to the bath, did not alter the responses to ACh in mouse ileum. As shown in Table 1, supersensitivity developed to ACh after 5 days of morphine treatment. However, the maximum response was not significantly altered. The supersensitivity was observed in all the tissues, irrespective of the presence of morphine in the bathing medium. This result supports the view of Rae & De Moraes (1983) that withdrawal does not have any role in the development of supersensitivity of the smooth muscle induced by chronic morphine treatment.

The daily sequential changes in the sensitivity of ACh in mouse ileum produced by morphine and in analgesia were investigated earlier with three dose regimens of morphine. The results revealed a positive correlation between the development of analgesic tolerance and supersensitivity to ACh in mouse ileum (Ramaswamy et al 1981). In the same study it was also found that

Table 1. Development of supersensitivity to acetylcholine after morphine.

Treatment	ED50 of ACh mean ± s.e.m. (ng)	Maximum response ± s.e.m. (mm)
Saline	34·25 ± 4·82	95·80 ± 6·90
†After 5 days morphine		
Without morphine in the bath	12·04 ± 1·86*	102·90 ± 4·80
With morphine (300 nM)	13·76 ± 2·56*	110·85 ± 7·20

† The animals were killed at 5 h after the last morphine treatment.
* $P < 0·01$ compared with saline value, n = 6.

naloxone at the end of 13 days morphine treatment enhanced the degree of ACh supersensitivity to 90 fold. These results and that observed in the present study clearly indicate that supersensitivity can be included as a reliable index to assess morphine tolerance and dependence. The inhibitory effect of morphine on ACh release in mouse ileum was suggested as a possible mechanism for morphine induced supersensitivity to ACh.

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Preliminary study of the disposition in man of acebutolol and its metabolite, diacetolol, using a new stereoselective hplc method

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A new stereospecific hplc method that is capable of simultaneously quantitating the *S*-(−)- and *R*-(+)-enantiomers of acebutolol and its major metabolite, diacetolol, in plasma and urine, is described. When applied to the assay of biological fluids collected during single and chronic oral dosing with acebutolol (Sectral), this procedure failed to reveal any important stereoselectivity in the disposition of either acebutolol or diacetolol in man. This may occur because acebutolol is metabolized by hydrolysis and *N*-acetylation, whereas the other β-blockers which exhibit some degree of stereoselective disposition (e.g. metoprolol and propranolol) are primarily metabolized by oxidation.

We have previously reported on the fate in man of the cardioselective β-adrenoceptor antagonist, acebutolol (Gabriel et al 1981). However, acebutolol and its major metabolite, diacetolol (the acetamido analogue), exist as *S*-(−)- and *R*-(+)-enantiomers, and, as with β-blocking drugs in general, most of the β-blocking activity resides in the *S*-(−)-enantiomer. For propranolol (Hermansson & Von Bahr 1980; Silber & Riegelman 1980; Tawara et al 1981; Silber et al 1982; Von Bahr et al 1982), alprenolol and metoprolol (Hermansson & Von Bahr 1982; Lennard et al 1983), bufuralol (Francis et al 1982) and moprolol (Harvengt & Desager 1982) there are higher circulating concentrations of the *S*-(−)-enantiomer compared with the corresponding *R*-(+)-enantiomer, and so we have re-examined the disposition of acebutolol in man by means of a new stereospecific hplc method.

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Because acebutolol has a major metabolite, diacetolol (Gulaid et al 1981), the stereospecific hplc method outlined below was designed to be capable of simultaneously quantitating the enantiomers of both acebutolol and diacetolol. The method involves the use of an optically pure derivatizing agent to form diastereoisomers, an approach previously described for other β-blocking drugs (see e.g. Hermansson & Von Bahr 1980 1982; Thompson et al 1982).

Method

The procedure is as follows: to each sample (1 ml) of plasma or urine is added 1 M NaOH (0·5 ml), the internal standard, M & B 17,764 which is the propionamido analogue of acebutolol (1 μg, as a concentrated methanolic solution), and a mixture (7 ml) of diethyl ether–chloroform (4 : 1 v/v), respectively. After having been shaken mechanically for 10 min the samples are centrifuged. The upper organic layer is removed and evaporated to dryness at about 60 °C under a stream of nitrogen (oxygen-free). To the dry residue is added chloroform (0·5 ml), triethylamine (10 μl) and the derivatizing agent (*S*-(−)-*N*-trifluoroacetylprolyl chloride (TPC), as a 0·1 M solution in chloroform; 100 μl). These are thoroughly mixed and then allowed to stand at room temperature (ca 20 °C) for about 25 min. To this solution is then added a mixture (7 ml) of diethylether–chloroform (4 : 1 v/v, respectively) followed by water (3 ml). After 10 min shaking and centrifugation, the upper organic layer is removed and evaporated as before. The dry residue is dissolved in