The role of interaction between opiate receptors and α-adrenergic receptors in tolerance and dependence

L. E. ROBSON, R. F. MUCHA* and H. W. KOSTERLITZ

UTC Unit for Research on Addictive Drugs, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland, U.K.

The development of tolerance and dependence as a consequence of prolonged administration of opiates is a well-known phenomenon, which has attracted the attention of pharmacologists for many years. Many theories proposed to explain the underlying mechanisms involve, as a pivotal point, alterations in the neurotransmitter systems of the endogenous opioid peptides. Our interest in a possible role of interactions between opiate and adrenergic receptors in tolerance and dependence was aroused by observations in the isolated electrically stimulated ileum from guinea pigs treated chronically with morphine (Goldstein & Schulz, 1973). Such preparations are ‘tolerant’, i.e. they showed considerably less sensitivity to the inhibitory effects of morphine than did ileum from control animals. In addition, however, the inhibitory effects of α-adrenergic-receptor agonists were diminished after the chronic morphine treatment.

In ileum isolated from guinea pigs implanted with two morphine pellets (150 mg) 3 days before the experiments, the inhibitory effects of the α1-adrenergic-receptor agonist clonidine were diminished and the dose–response curves were flat (Gillan, et al., 1979a). When morphine was present from the bath fluid at the time of testing, the preparations were considered to be in a ‘withdrawn’ state analogous to that which would occur in the whole animal after removal of the pellets. Emulation of the ‘dependent’ state, analogous to the situation in the whole animal before pellet removal, was accomplished by addition of morphine to the bath fluid; the inhibitory effect of clonidine was restored. This interaction between the opiate receptor and α1-adrenergic-receptor agonists had been induced by the chronic morphine treatment, since clonidine-sensitivity in ileum from non-implanted guinea pigs was not changed by adding morphine to the bath fluid. The inhibitory effects of other α1-adrenergic-receptor agonists were also changed after implantation of morphine pellets, as a similar pattern of effects was seen when oxytetracyclorol was used instead of clonidine. However, when adrenaline was used, the diminution in the inhibitory effects in the ‘withdrawn’ state were seen only at low concentrations, and not at high ones; addition of morphine to the bath fluid restored the inhibitory effects of adrenaline. A pattern of responses similar to that of adrenaline was seen when the effects of adenosine 3',5'-diphosphate or 6-furfurylaminopurine riboside were seen when clonidine was absent from the bath fluid. The first question that arises concerns the site of such interactions between opiate receptors and α-adrenergic receptors in the guinea-pig ileum. Investigations of possible changes at the recognition site of the opiate receptor after chronic morphine treatment have produced inconsistent results (Klee & Streayt, 1974; Davis et al., 1975; Cox & Padhya, 1977; Oishi & Takemori, 1982). As far as possible changes at the recognition site of the α1-adrenergic receptor are concerned, it has been shown that the number of binding sites for [3H]clonidine are increased in homogenates of rat cerebral cortex or brain-stem after chronic treatment with morphine (Hamburg & Tallman, 1981). Nevertheless, since the presynaptic opiate receptors of the myenteric plexus are selectively antagonized by naloxone, and the presynaptic α1-adrenergic receptors by phenolamine or yohimbine (Kosterlitz & Watt, 1968; Malta et al., 1981), it is likely that the interaction occurs at a point between the recognition sites of the two receptors and the mechanism responsible for the release of the transmitter acetylcholine.

Although information on post-recognition processes is, as yet, somewhat limited, there is evidence that cyclic nucleotides may be involved, at least partly, in mediating the actions of opiates. For instance, stimulation of cyclic AMP formation by prostaglandins and noradrenaline inhibit basal and prostaglandin-stimulated adenylate cyclase activity to a significant extent, and this effect is mimicked by clonidine (Sharma et al., 1975). Activation of α1-adrenergic receptors of NG 108-15 cells also results in inhibition of adenosine uptake (Sabol & Nirenberg, 1979). The observation that the effects of concentrations of noradrenaline or norepinephrine, which give at least 90% of their maximal effect, are not additive suggests that the two receptors are coupled to the same pool of adenylyl cyclase activity.

In addition to the readily reversibly morphine-induced inhibition of adenylyl cyclase activity observed in NG 108-15 cells, a second process mediated by opiate receptors has been observed (Sharma et al., 1975b). In cells cultured in the presence of morphine, the basal and prostaglandin-stimulated adenylyl cyclase activities were at first inhibited but then recovered to normal levels (Sabol & Nirenberg, 1979b). These events have been suggested to be related to the phenomena of tolerance and dependence. Some support for this view is obtained from observations that cyclic AMP or phosphodiesterase inhibitors intensified some aspects of the opiate withdrawal syndrome (Ho et al., 1975; Collier & Francis, 1975), and, when administered to opiate-naive animals that were then treated with naloxone, produced some behavioural responses that are typically observed as part of the abstinence syndrome (Collier et al., 1974).

The relevance of the cell-culture system to the central and peripheral nervous system has still to be established. However, if similar changes may be assumed to occur in the myenteric plexus of the ‘dependent’ ileum, then an increase in the adenylyl cyclase activity or a change in the concentrations of other regulatory molecules might lead to such an increase in electrically evoked acetylcholine release that stimulation of the presynaptic α1-adrenergic receptors would be less effective. The findings presented here would be compatible with this interpretation, provided that it may be assumed that the presynaptic opiate receptors and α-adrenergic receptors share a common post-recognition mechanism in the guinea-pig ileum.

*B Present address: The Psychological Laboratory, University of Cambridge, Cambridge CB2 3EB, U.K.
The decreased sensitivity to clonidine would appear to be an important and reversible sign of withdrawal in the guinea-pig ileum. Such interactions between opiate receptors and α-adrenergic receptors do not appear to occur in the mouse vas deferens (Gillian et al., 1979). However, vasa deferentia from rats treated chronically with clonidine showed lower sensitivity to the inhibitory effects of β-endorphin and adenosine, as well as to those of clonidine (Ishii et al., 1982). In man, clonidine ameliorated certain aspects of the opiate withdrawal syndrome (Gold et al., 1978). It is possible that co-operation between the effects of administered clonidine and the residual opiate in centrally situated neurons may be the basis of this effect. Similarly, changes in a common presynaptic post-re cognition pathway may be related to the observation that, after chronic treatment of rats with clonidine, tolerance to the analgesic effect of clonidine is accompanied by cross-tolerance to morphine (Paalzow, 1978). Alternatively, in this latter instance, a common pathway mediating the analgesic effects of the compounds may be post-synaptic.

In ileum from morphine-treated guinea pigs, a contraction of the longitudinal muscle seen in response to naloxone is due, at least in part, to the release of acetylcholine from post-ganglionic neurones, and has been suggested to be related to the occurrence of withdrawal (Ehrenpreis et al., 1975; Schulz & Herz, 1976). However, the magnitude of the naloxone-induced contraction was the same in ileum treated with morphine in vitro under various regimes and in ileum from pellet-implanted animals. In contrast, the degree of depression of the response to clonidine varied, being most marked in tissues from pellet-implanted animals. The relationship between opiate-tolerance and -dependence is obscured by the fact that tolerance cannot be measured without giving drug, and dependence without withdrawing it (Collier, 1973). Some part of opiate-tolerance appears, however, to be closely associated with dependence (Wüster, 1982); and so we can speak of opiate-dependence and associated tolerance.

Opiate-dependence and associated tolerance was first observed in man and later reproduced in laboratory animals. More recently the essential features of this dependence and tolerance were reproduced in normal neurones in vitro (Ehrenpreis et al., 1972; Hammond et al., 1976; Villarreal et al., 1977; Lujan & Rodriguez, 1981; Collier et al., 1981b) and in cultures of isolated malignant hybrid cells of mammalian neural origin (Sharma et al., 1975). These findings show that opiate-dependence and associated tolerance need not be an integrated response of a neuronal system but can be a cellular phenomenon developing within cells that bear opiate receptors (North & Karras, 1978; Collier, 1978, 1980). These models provide a practical means of analysing the phenomenon in vitro.

We have studied opiate-dependence and associated tolerance in the final cholinergic motor neurones of the myenteric plexus supplying the longitudinal smooth muscle of the guinea-pig ileum. The predominant opiate receptor in this preparation is of the α subtype, though ρ-receptors have been demonstrated (Gillian et al., 1980; Schulz et al., 1981). The ρ-receptor is particularly responsive to morphine, normorphine and naloxone. When the isolated guinea-pig ileum is exposed to repeated electrical stimulation, the longitudinal smooth muscle contracts in response to the release of acetylcholine from the terminal of the final cholinergic motor neurones. Opiates inhibit this response in a dose-related manner (Paton, 1957; Schaumann, 1957). If exposure to opiate is prolonged, the inhibitory effect develops tolerance (Paton, 1957; Fennessy et al., 1969). If, after prolonged exposure to opiate, the non-electrically stimulated preparation is challenged with naloxone, the longitudinal muscle contracts sharply in response to release of acetylcholine from the terminals of the final cholinergic motor neurones (Ehrenpreis et al., 1972).


### Adaptation of a neuron to normorphine and clonidine

**NIGEL J. CUTHBERT, DAVID L. FRANCIS and HARRY O. J. COLLIER**

**Dome/Hollister–Stier Research Centre, Stoke Court, Stoke Poges, Slough, Berks. SL2 4LY, U.K.**

Continued treatment of a mammal with opiate induces tolerance and dependence. Tolerance is characterized by a diminished response to opiates, and dependence by (i) a heightened response to selective opiate antagonists and (ii) a behavioural disturbance on withdrawal (the withdrawal syndrome). The relationship between opiate-tolerance and -dependence is obscured by the fact that tolerance cannot be measured without giving drug, and dependence without withdrawing it (Collier, 1973). Some part of opiate-tolerance appears, however, to be closely associated with dependence (Wüster, 1982); and so we can speak of opiate-dependence and associated tolerance.

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Fig. 1 illustrates our method of inducing and measuring opiate-dependence and associated tolerance (Hammond et al.,...