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 (Gillan 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-recognition 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 postsynaptic.

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 nalozone-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. It would therefore appear that the two phenomena are not related in a simple way.

This work was supported by grants from the U.K. Medical Research Council and U.S. National Institute of Drug Addiction (DA00662). R. F. M. holds a fellowship of the Canadian Medical Research Council.


### 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.

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 neurons 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 μ-subtype, though κ-receptors have been demonstrated (Gillan et al., 1980; Schulz et al., 1981). The μ-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).

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

BIOCHEMICAL SOCIETY TRANSACTIONS

Pieces from the same ileum placed in Krebs solution containing hexamethonium with or without opiate and/or other test drug. Incubated at 5°C or 22°C for 18–24 h or at 37°C for 8 h.

Set up in incubation fluid at 37°C in parallel organ baths for recording responses to added drugs or electrical stimuli.

Tolerance: shift to right of D/R line for inhibition of electrically evoked contracture of ileum. N₁, lower, and N₂, higher dose of normorphine.

Dependence: dose-related contracture of opiate-incubated preparations to naloxone (Nₓ) compared with standard dose of acetylcholine (ACh)

Fig. 1. Method of inducing and recording opiate-tolerance and dependence in guinea-pig ileum in vitro (Collier, 1982)

1976; Collier et al., 1981) in the isolated ileum. By using this procedure, we have established seven characteristics of opiate-dependence and associated tolerance in the ileum. They are: (1) dependence is accompanied by tolerance to the acute effect of opioids; (2) dependence develops only after exposure to opiate; (3) withdrawal of opiate elicits a contracture that can be suppressed by opiate; (4) the withdrawal contracture can be precipitated with a selective opiate antagonist, the potency of which is inversely related to the intensity of dependence; (5) induction of dependence requires the activation of a stereospecific opiate receptor; (6) precipitation of withdrawal contracture requires a stereospecific opiate antagonist; (7) clonidine inhibits the opiate-withdrawal contracture. These properties of opiate-dependence and associated tolerance induced in the ileum in vitro parallel those observed in the whole animal.

Dependence on the α-adrenergic-receptor agonist clonidine, like that on opiate, has been reported in experimental animals (Meyer et al., 1977; Jenn repeated et al., 1980) and in man (Hansson et al., 1975; Reid et al., 1977). Sabol & Nirenberg (1979) showed that clonidine induces dependence in cultured neuroblastoma x glioma hybrid cells. Like opiates, clonidine, acutely, inhibits the electrically evoked release of acetylcholine from the final cholinergic motor neurons of the ileum. 

The application of phentolamine, a selective α₁-adrenergic-receptor antagonist, to segments of ileum that had been continuously exposed to clonidine as in Fig. 1, induced a release of acetylcholine resulting in a contracture of the ileum (Collier et al., 1981). This contracture was taken to indicate a state of withdrawal from clonidine, since it exhibited most of the seven characteristics of opiate-dependence and associated tolerance seen in the ileum. Of these, tolerance was demonstrated indirectly by the comparability of the responses to electrical stimulation in the presence and in the absence of clonidine after 24 h incubation. A spontaneous withdrawal contracture, however, was not seen, probably because the binding of clonidine to its recognition site is prolonged.

There is evidence that both opiate-dependence and clonidine-dependence are induced in the ileum in vitro. The induction of dependence on clonidine in the ileum in vitro.

1983
hexamethonium sufficient to block descending impulses from cholinergic neurons supplying the final cholinergic motor neurons (Hammond et al., 1976; North & Karras, 1978; Collier et al., 1981a,b). Tetrodotoxin, which prevents acetylcholine release from the terminals of the final cholinergic motor neurons, and hyoscine or atropine, which blocks the acetylcholine receptor on the smooth muscle, both suppress the contractile response to challenge with naloxone in opiate-dependence and associated tolerance (Ehrenpreis et al., 1972; Schulz & Herz, 1976; North & Karras, 1978; Collier et al., 1981b) and with phentolamine in clonidine-dependence (Collier et al., 1981a). Furthermore, the acute inhibitory effects of opiate and clonidine have been localized to the final cholinergic motor neurons of the myenteric plexus (Paton & Aboozar, 1968; Greenberg et al., 1970; Kromeberg & Oberdorf, 1971). Again, cholinergic neuron suppresses the naloxone-induced contracture in opiate-dependence and opiate suppresses the phentolamine-induced contracture in clonidine-dependence preparation.

Having established that a dependence mechanism, which may be activated by two separate classes of inhibitory transmitter, occurs in the final cholinergic motor neurons, can we go further and identify the region of the neuron that is involved? Fig. 2 illustrates the regions of the final cholinergic motor neuron along with the receptors that may be present.

The first possible region to consider is the recognition site of the receptor. That drugs interacting with two different recognition sites (opiate receptor and a-adrenergic receptor) induce comparable dependences indicates that these occur downstream of the recognition sites. This is supported by the observation by Schulz et al. (1981) on selective agonists of opiate-μ and κ-receptors.

We have as yet no evidence that dependence in the final cholinergic motor neuron occurs in the axon or nerve terminal; but there is good evidence that it develops in the soma. North & Karras (1978) and Karras & North (1981), using suction electrodes attached to the soma of the final cholinergic motor neuron, have investigated nerve-impulse production in response to treatment with opiates. They found that neurons, either derived from guinea pigs treated with high doses of morphine for several days or in pieces of ileum incubated with opiate in vitro, were less responsive to inhibition by normorphine than were controls. These neurons also responded vigorously to challenge with naloxone by generating impulses at higher rates than did control neurons. This evidence suggests that the change that occurs during induction of dependence does so in the soma of the final cholinergic motor neuron.

The molecular mechanism of the induction of dependence in the soma has not, as yet, been further elucidated, but some interesting work in other systems is relevant. One such system consists of cultures of mouse neuroblastoma x rat glioma hybrid cells strain NG 108–15. These cells are neuron-like in that they form action potentials on stimulation and possess opiate receptors (Klee & Nirenberg, 1974) and a-adrenergic receptors (Sabol & Nirenberg, 1979). The application of either opiate or a-adrenergic-receptor agonist to these cells results in an inhibition of adenylate cyclase activity (Traber et al., 1975; Sharma et al., 1975a; Sabol & Nirenberg, 1979). Koski & Klee (1981) says that opiates achieve this inhibition by stimulating the hydrolysis of GTP, which is itself an activator of adenylate cyclase. Therefore the immediate effect of opiate is to lower the cellular concentration of cyclic AMP. Prolonged exposure to opiate results in a compensatory increase in adenylate cyclase activity to restore 'normal' concentrations of cyclic AMP. This intensified enzyme activity produces tolerance, and, if opiate is withdrawn by applying naloxone or clonidine is withdrawn by applying phentolamine, a burst of cyclic AMP production occurs (Sharma et al., 1975b, 1977; Lampert et al., 1976; Sabol & Nirenberg, 1979).

Although there is no direct evidence that the involvement of a hypertrophy of the cyclic AMP system being the mechanism of opiate-dependence in normal neurons, there is indirect evidence from whole animals that this is so (Collier & Roy, 1974; Francis et al., 1975; Collier, 1980).

Deficiency of chronic amphetamine administration on dopaminergic systems in the vervet brain: Relationship to findings in the brains of schizophrenics

FRANK OWEN, HARRY F. BAKER, ROSALIND M. RIDLEY, ALAN J. CROSS and TIMOTHY J. CROW
Division of Psychiatry, Clinical Research Centre, Watford Road, Harrow, Middx. HA1 3UJ, U.K.

It has been well established that amphetamines can induce a schizophrenia-like psychosis in mentally normal individuals (Connell, 1958; Griffiths et al., 1972; Bell, 1973). As far as an adjunct to our biochemical studies on post-mortem brains of schizophrenics (Crow et al., 1978; Owen et al., 1978) we have studied neurotransmitter-related biochemistry in brains of vervet monkeys after prolonged amphetamine administration. Because of the strong evidence implicating excessive central dopaminergic function in the aetiology of schizophrenia (see Crow et al., 1979) and the pronounced effect of amphetamine on dopaminergic mechanisms (Moore, 1977), our initial studies in vervet brain were concerned with an evaluation of dopamine (3,4-dihydroxyphenethylamine) metabolism.

Five previously undrugged vervets received (+)-amphetamine sulphate in increasing doses in their drinking water for 35 days as depicted in Fig. 1. Four, similarly housed, untreated vervets served as controls. At the end of this time the animals were deeply anaesthetized and the brains were removed for the biochemical analyses. Dopamine was measured by the radioimmunoassay technique of Coyle & Henry (1973), and 3,4-dihydroxyphenylacetic acid and homovanillic acid were determined by high-pressure liquid chromatography (Cross & Joseph, 1981). Tyrosine hydroxylase activity was assayed by the method of Lerner et al. (1977), and 3,4-dihydroxyphenylalanine decarboxylase activity by the method of McCammon et al. (1972).

Dopamine receptors were assessed as described by Owen et al. (1978). The results in the three major dopaminergically innervated areas of the brain, namely caudate, putamen and nucleus accumbens, are presented in Table 1. For clarity all results are expressed as percentages of their respective controls.

Table 1. Biochemical results in vervet brains after prolonged administration of amphetamine

<table>
<thead>
<tr>
<th>Concentration or activity (%) of control value</th>
<th>Caudate</th>
<th>Putamen</th>
<th>Nucleus accumbens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>7.1***</td>
<td>12.8**</td>
<td>25.2*</td>
</tr>
<tr>
<td>3,4-Dihydroxyphenylacetic acid</td>
<td>14.5***</td>
<td>13.3***</td>
<td>39.4***</td>
</tr>
<tr>
<td>Homovanillic acid</td>
<td>74.8*</td>
<td>90.6</td>
<td>68.0*</td>
</tr>
<tr>
<td>Tyrosine hydroxylase</td>
<td>32.3*</td>
<td>38.9**</td>
<td>—</td>
</tr>
<tr>
<td>Dopa decarboxylase</td>
<td>42.0*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>[3H]Spiroperone binding</td>
<td>78.9</td>
<td>80.6</td>
<td>52.9</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01; ***P < 0.001 (Student's t test).

Fig. 1. Amphetamine dosage schedule of vervets over 35-day period.

Amphetamine (mg/kg per day) 4 6 8 10 12
Duration (days) 0 5 10 15 20 25 30 35

| Concentration or activity (%) of control value |
|-----------------------------------------------|---------|---------|------------------|
| Caudate | Putamen | Nucleus accumbens |
| Dopamine | 7.1***  | 12.8**  | 25.2*            |
| 3,4-Dihydroxyphenylacetic acid | 14.5*** | 13.3*** | 39.4***          |
| Homovanillic acid | 74.8*   | 90.6    | 68.0*            |
| Tyrosine hydroxylase | 32.3*   | 38.9**  | —                |
| Dopa decarboxylase | 42.0*   | —       | —                |
| [3H]Spiroperone binding | 78.9    | 80.6    | 52.9             |

1983