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CLONIDINE DEPENDENCE IN THE GUINEA-PIG ISOLATED ILEUM

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1 Compared with the response of preparations incubated in solutions without clonidine, a three to four fold increase in the magnitude of the contracture of the longitudinal muscle to challenge with phentolamine (1.0 μM) was induced by incubating the guinea-pig isolated ileum at 22°C for 24 h with clonidine (1.0 μM) in Krebs solution containing hexamethonium (70 μM). Incubation of the ileum with clondine (1.0 μM) for 0.5 h at 37°C did not increase responsiveness to phentolamine.

2 The increase in responsiveness to phentolamine was directly related to the clonidine concentration in the incubation fluid over the range 0.01 to 1.0 μM.

3 The magnitude of the contracture to phentolamine of ilea incubated with clonidine (1.0 μM) (withdrawal contracture) was directly related to the challenge dose of phentolamine over the range 0.3 to 1.0 μM.

4 Yohimbine (1.0 μM) or piperoxane (1.0 μM) elicited a response comparable to that elicited by phentolamine but propranolol (1.0 μM) was inactive.

5 Addition of phentolamine (1.0 μM) to clonidine (1.0 μM) in the incubation fluid abolished the increased response of the preparation to subsequent challenge with phentolamine.

6 Addition of hyoscine (0.5 μM) immediately after challenge with phentolamine restored the tension of the withdrawal contracture to its resting level.

7 Tetrodotoxin (3.0 μM) given before challenge, prevented phentolamine from eliciting a withdrawal contracture.

8 Ileal segments incubated with clonidine (1.0 μM) were unresponsive to challenge with naloxone (100 nM); and segments incubated with normorphine (1.0 μM) were unresponsive to phentolamine (1.0 μM), although responsive to naloxone.

9 Normorphine (1.0 μM) restored to resting level the tension of the clonidine withdrawal contracture; and clonidine (0.1 μM) restored to resting level the tension of the contracture to naloxone in ileal segments incubated with normorphine.

10 These experiments indicate that incubation with clonidine induces, in the final cholinergic motor neurones of the myenteric plexus of the isolated ileum, a dependence the withdrawal from which is expressed as a contracture in response to α-adrenoceptor antagonists.

11 Although opiate receptors are not involved in clonidine dependence nor α-adrenoceptors in opiate dependence, the findings that normorphine suppresses the clonidine withdrawal-contracture and that clonidine suppresses the contracture of opiate-dependent ileum to naloxone, suggest that the withdrawal effect studied in both clonidine and normorphine dependence in this preparation is mediated by release of acetylcholine from the final motor neurone.

Introduction

Physical dependence on clonidine, expressed as hyperactivity or hypertension after withdrawal of the drug, has been described in experimental animals (Meyer, El-Azhary, Bierer, Hanson, Robbins & Sparber, 1977; Jennewein, Stockhaus & Hoefke, 1980) and in man (Hansson, Hunyor, Julius & Hoobler, 1973; Reid, Dargie, Davies, Wing, Hamilton & Dollery, 1977; Geyskes, Boer & Dorhout-Mees, 1979). In the whole animal, many variables confuse the analysis of the molecular mechanisms underlying dependence, which requires models of clonidine dependence in isolated cells or organs. One such model is provided by cultured neuroblastoma X glioma hybrid cells, which show an increased responsiveness of their adenylate cyclase to α-adrenoceptor antagonists, after incubation with α-adrenoceptor agonists (Sabol & Nirenberg, 1979). However, these cells are not normal neurones. We have therefore sought a method of inducing dependence on clonidine in normal neurones *in vitro*.

Dependence on opiates, expressed as an increased responsiveness to a specific antagonist, or as increased activity on withdrawal of the opiate, can be induced *in vitro* by incubating isolated segments of

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guinea-pig ileum with opiate (Ehrenpreis, Light & Schonbuch, 1972; Hammond, Schneider & Collier, 1976; Villarreal, Martinez & Castro, 1977; North & Karras, 1978; Collier, Cuthbert & Francis, 1980a,b). Clonidine, an α-adrenoceptor agonist, shares with opiates the ability to reduce the release of acetylcholine from the final motor neurones of the myenteric plexus of the guinea-pig isolated ileum (Kroneberg & Oberdorf, 1971; Deck, Oberdorf & Kroneberg, 1971; Gillan, Kosterlitz, Robson & Waterfield, 1979; Tanaka & Starke, 1979; Tayo, 1979) and has been shown to reduce the effects of opiate withdrawal in experimental aminals (Tseng, Loh & Wei, 1975; Vetulani & Bednarczyk, 1977; Aghajanian, 1978; Fielding, Wilker, Hynes, Szewczak, Novick & Lal, 1978; Sparber, & Meyer, 1978; Crawley, Laverty & Roth, 1979; Laverty & Roth, 1980) and man (Gold, Redmond & Kleber, 1978; Gold, Pottash, Sweeney & Kleber, 1979; Washton, Resnick & Rawson, 1979; Riordan & Kleber, 1980). We have therefore explored the possibility of using the ileum as a model of clonidine dependence *in vitro*.

We describe below (a) experiments in which isolated segments of guinea-pig ileum, incubated with clonidine, show an increased responsiveness to specific α-adrenoceptor antagonists, which is interpreted as clonidine dependence, (b) some of the properties of this dependence and (c) some experiments exploring the relationship of clonidine- to opiate-dependence in the isolated ileum. Some of these results have been presented to the British Pharmacological Society (Collier, Cuthbert & Francis, 1980c).

Methods

Preparation of ileal segments

Male Dunkin-Hartley guinea-pigs (A.J. Tuck & Sons) weighing 300–400 g were killed by cervical dislocation. Pieces of ileum about 70 cm long were immediately removed from a point 10 cm oral to the ileo-caecal junction. These pieces were placed in modified Krebs solution containing hexamethonium (70 μM), flushed through with the same solution and then divided into the desired numbers of 10 cm segments. Segments were taken first from the aboral end and randomized between treatments. Control and test segments were prepared for incubation in sets of 2–6 from the same animal.

Incubation procedure

An incubation temperature of 22°C was used throughout the present series of experiments because this temperature had been found satisfactory in the induction of opiate dependence in the same preparation (North & Karras, 1978; Collier *et al.*, 1980a, b). To allow as much opportunity as possible for dependence to develop, ilea were incubated for 24 h. Despite regular changes of incubation fluid, preparations incubated for this period at 22°C tended to show greater variation in baseline and in magnitude of their response to acetylcholine (ACh) and to electrical stimulation than do fresh preparations, as the representative tracings illustrated show. Nonetheless, in all preparations used, the responses to ACh and to electrical stimulation had essentially the same shape and time characteristics as did those of fresh preparations.

Segments of ileum were incubated for 24 h at room temperature (22 ± 2°C) in 10 ml baths containing pre-gassed (95% O_2 and 5% CO_2) incubation fluid (modified Krebs solution containing 70 μM hexamethonium) with or without clonidine and/or other test substance. The 10 ml incubation baths were connected to large reservoirs containing incubation fluid which was continuously gassed. The tubing connecting the baths to the reservoirs passed through the rollers of a multi-channel infusion pump that, when operated for a 5 min period at 30 min intervals, completely changed the bath contents. The bath overflow went to waste via a vacuum line. The reservoirs and incubation baths were shielded from light.

Determination of dependence

Unless otherwise stated, the following test procedure was used. Two to four segments of ileum from the same animal, but incubated under different conditions or to be tested under different conditions, were set up in parallel for comparative tests. Ilea were set up for transmural stimulation in 40 ml organ baths at 37°C (Gyang & Kosterlitz, 1966; Hammond *et al.*, 1976). The bath solution, which was equivalent to that used for incubation, was continuously gassed (95% O_2 and 5% CO_2). A negative pressure of 1 cm saline was applied to the aboral end of the tissue to remove excess mucus. When appropriate, the ileum was stimulated transmurally, using a Grass S88 stimulator set to give 40 V (supramaximal) square wave pulses of 0.5 ms duration, at 0.1 Hz. Contractions were recorded isometrically with a Statham Gold Cell transducer and a Devices potentiometric recorder.

After 30 min equilibration with washes at 15 min intervals, a tension of 1 g was applied to the transducer for calibration purposes. Four minutes later, 4–6 electrical pulses were given, followed by a wash. Five minutes later, the challenge dose of phentolamine, other test drug, or vehicle was applied and left in contact with the tissue for 2 min. This was followed, after washing, by challenge with ACh as either a single dose (10 nM) or a series of discrete ascending doses (0.01, 0.1, 1.0, 3.0, 10 μM) until the

maximal contraction of the longitudinal muscle had been reached. To allow for variability between tissues in responsiveness (maximal and submaximal) to ACh, response to antagonist challenge was expressed as a ratio of the peak tension elicited by antagonist to that elicited by 10 nM ACh (tension ratio); an analysis of variance of this variability showed that it was unrelated to treatment.

Statistical analysis of the significance of differences between values from comparable pairs of preparations was performed by Student's *t* test. Slopes were determined by least squares linear regression analysis.

Materials

Substances used were acetylcholine iodide (Sigma), clonidine (Boehringer-Ingelheim) hexamethonium bromide (Sigma), hyoscine hydrochloride (Sigma), naloxone hydrochloride (Endo), normorphine (Wellcome), phentolamine hydrochloride (Ciba), piperoxane hydrochloride (May & Baker), propranolol hydrochloride (Imperial Chemical Industries), tetrodotoxin (Sigma) and yohimbine hydrochloride (Sigma). Normorphine was dissolved in 0.3 ml of 0.5 N HCl and made up to volume with distilled water. All other drugs were dissolved in distilled water. Concentrations given for each drug refer to the final bath concentration. No drug was applied to the bath in a volume exceeding 0.4 ml. The incubation fluid was modified Krebs of the following composition (mM): NaCl 117.5, KCl 4.75, $CaCl_2$ 2.6, KH_2PO_4 1.19, $MgSO_4$ 1.2, $NaHCO_3$ 24.5 and glucose 11; it also contained hexamethonium, 70 μM.

Results

Induction of clonidine dependence

Segments of ileum, taken in pairs from the same

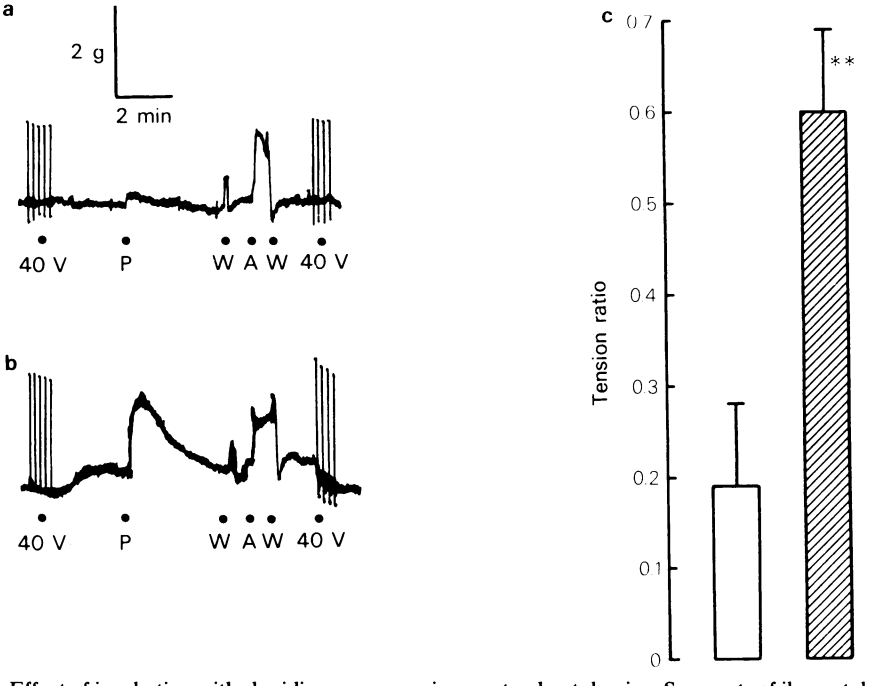

Figure 1 Effect of incubation with clonidine on responsiveness to phentolamine. Segments of ileum, taken in pairs from the same animal, were incubated for 24 h at 22°C in 10 ml baths containing pre-gassed incubation fluid (modified Krebs solution containing 70 μM hexamethonium) with or without clonidine (1.0 μM). After incubation the ileal segments were set-up, as for transmural stimulation, at 37°C in 40 ml baths and allowed to equilibrate for 0.5 h. After 4–6 pulses (40 V) 1 g tension was applied to calibrate the recording system. This was followed by challenge with phentolamine (1.0 μM) (P), and, after several washes (W), by a single challenge with acetylcholine (ACh) 10 nM (A), or challenge with an ascending series of doses of ACh (0.01–10 μM) until the maximal contracture was elicited. Tracings show the effect of phentolamine challenge on a pair of segments incubated without (a) or with clonidine 1.0 μM (b), to which a single challenge of ACh (10 nM) was given; (c) shows mean responsiveness to phentolamine (1.0 μM), expressed as a ratio of the maximum tension elicited by phentolamine to that elicited by 10 nM ACh (tension ratio) in 10 pairs of segments incubated with (hatched column) or without (open column) clonidine (1.0 μM). Vertical bars show the s.e.mean. Significance values were determined using Student's *t* test: $^{**}P<0.01$.

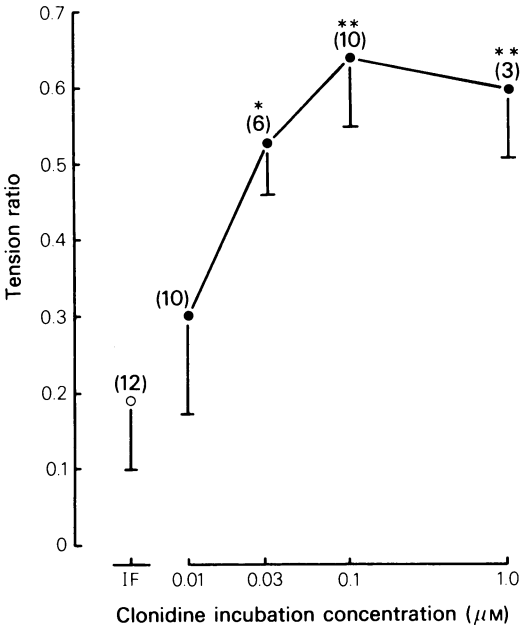

Figure 2 Effect of incubation concentration of clonidine on responsiveness to phentolamine. Segments of ileum, taken in sets of three from the same animal were incubated in incubation fluid (IF) alone (○) or in this fluid with the addition of 0.01, 0.03, 0.1 or 1.0 μM clonidine (●). The tension ratio expresses the contracture elicited by phentolamine (1.0 μM). Points give the mean and vertical bars the s.e.mean of at least 3 experiments (number in parentheses). For significance of difference from segments incubated without clonidine: $*P<0.05$; $**P<0.01$. Other details as in Figure 1.

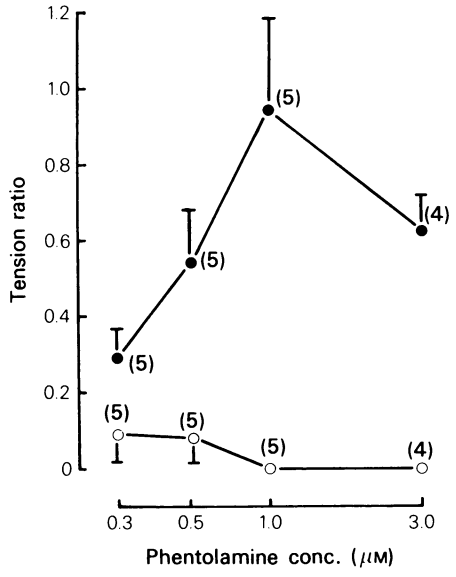

Figure 3 Effect of challenge concentration of phentolamine on the extent of the contracture elicited from segments of ileum incubated with clonidine (1.0 μM). Other details as in Figures 1 and 2.

animal, were incubated in the incubation fluid with or without the addition of 0.01, 0.1 or 1.0 μM clonidine. Figure 1, which gives a pair of tracings from a typical experiment and the mean results of ten experiments, shows that challenge with phentolamine (1.0 μM) elicited a strong contracture in ileal segments incubated with 1.0 μM clonidine, but had only a slight effect in segments not exposed to clonidine ($P<0.01$). Responses to electrical stimulation and to subsequent challenge with ACh were not affected by incubation with clonidine. Control experiments showed that phentolamine (1.0 μM) elicited a measurable contracture, the mean tension ratio being 0.41 ± 0.23 g (n = 12), in ileal segments that had been incubated for 24 h in the control incubation fluid, whereas this dose of phentolamine failed to elicit any contracture in ileal segments from freshly killed guinea-pigs ($n = 4$) or in ileal segments ($n = 4$) that had been exposed to clonidine (1.0 μM), for 0.5 h at 37°C.

The increase in responsiveness of segments incubated with clonidine to challenge with phentolamine was directly related to the concentration of clonidine in the incubation fluid (Figure 2). Over the dosage range 0.01–0.1 μM, the slope was 0.34 ± 0.16 ($P<0.05$). Incubation with clonidine at these concentrations did not significantly affect the sensitivity of the longitudinal muscle to challenge with a low dose of ACh (10 nM), or to the maximal contracture elicited by higher doses of ACh.

Figure 3 shows that, over the dose-range of 0.3–1.0 μM, the magnitude of the contracture elicited by challenge with phentolamine in ileal segments incubated for 24 h with clonidine (1.0 μM) was directly related to the dose of phentolamine (slope 1.26 ± 0.44; $P<0.05$). Doses of phentolamine above 1.0 μM did not produce a greater effect.

The α-adrenoceptor antagonists, yohimbine and piperoxane (each at 1.0 μM) also elicited a pronounced contracture in segments of ileum that had been incubated for 24 h in clonidine (1.0 μM). The mean tension ratios were: yohimbine, 0.33 ± 0.06 ($n = 4$) and piperoxane, 0.26 ± 0.06 ($n = 4$). These antagonists elicited little or no contracture in ileal segments incubated without clonidine, when the mean tension ratios were: yohimbine, 0.12 ± 0.04 ($n = 4$; $P<0.05$); piperoxane, 0 ($n = 4$).

If the increase in responsiveness to phentolamine were induced via an interaction between clonidine and specific α-adrenoceptors, then incubation with clonidine should not induce an increase in responsiveness to β-adrenoceptor antagonists, such as prop-

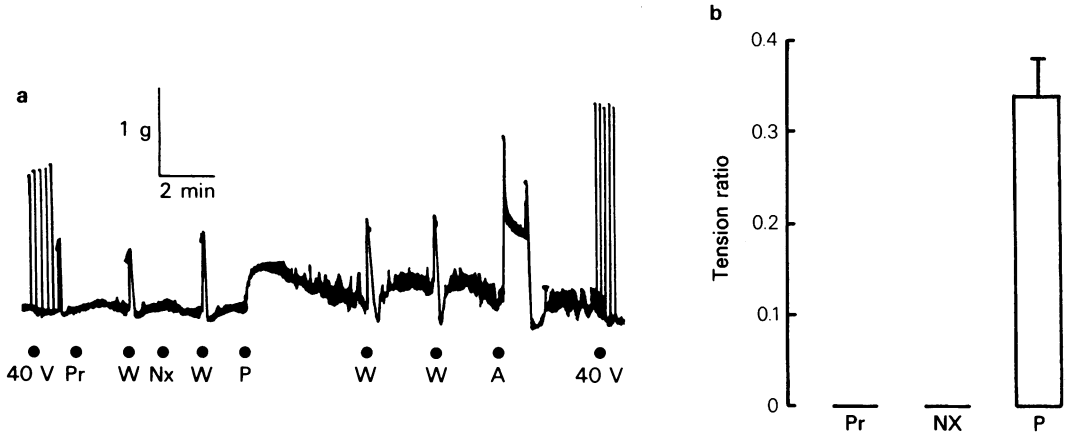

Figure 4 Effects of challenge with propranolol, naloxone or phentolamine on ileum incubated with clonidine. Segments of ileum were incubated for 24 h at 22°C in incubation fluid containing clonidine (1.0 μM). After being set up for test, the ileal segments were challenged successively with propranolol 1.0 μM (Pr), naloxone 0.1 μM (Nx) and phentolamine 1.0 μM (P). Challenges were given at 3 min intervals with a wash (W) between challenges. Columns (b) show the mean tension ratio ($n = 4$) and vertical bars the s.e.mean. Other details as in Figures 1 and 3.

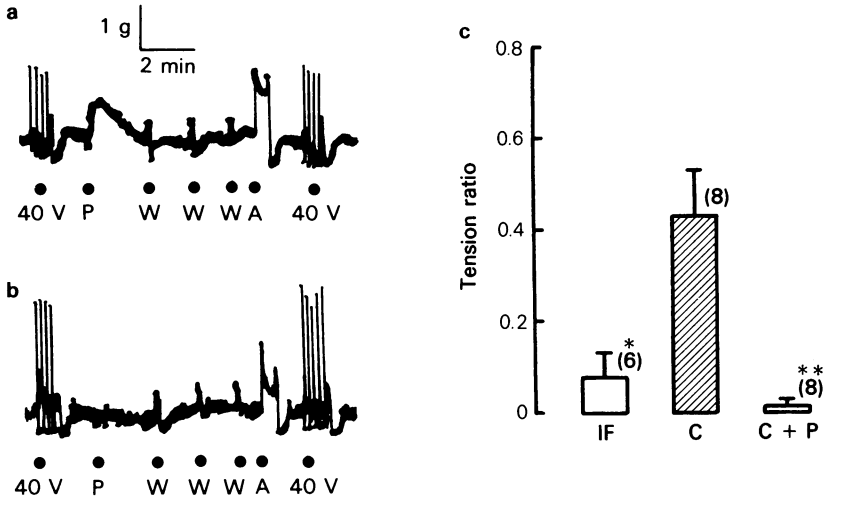

Figure 5 Effect of phentolamine in the incubation fluid on the induction by clonidine of responsiveness to phentolamine challenge. Segments of ileum, taken in threes from the same animal, were incubated in fluid containing either (i) clonidine 1.0 μM (a and column C, in c); (ii) clonidine 1.0 μM plus phentolamine 1.0 μM (b and column C+P in c) or (iii) no addition (column IF, in c). After incubation, each tissue was set up for test as described in Figure 1, and challenged with phentolamine 1.0 μM (P). Columns (c) give the mean tension ratio and vertical bars give the s.e.mean of at least six experiments (numbers in parentheses). Significance of difference from tissues incubated with clonidine: $*P<0.05$; $**P<0.01$. Other details as in Figure 1.

ranolol. Figure 4 shows a typical tracing and mean results from four experiments in which propranolol (1.0 μM) did not elicit a contracture in ileal segments that had been incubated with clonidine (1.0 μM). These segments were, however, highly responsive to challenge with phentolamine (1.0 μM).

If the increased responsiveness to phentolamine were induced through an interaction between clonidine and specific α-adrenoceptors, then the extent of contracture should be reduced by agents competing with clonidine for these receptors. To test this, segments of ileum taken in threes from the same animal were incubated in medium containing either (a) clonidine (1.0 μM), (b) clonidine (1.0 μM) plus phentolamine (1.0 μM) or (c) no addition. Figure 5 shows that, whereas ileal segments incubated with clonidine were highly responsive to challenge with phentolamine (1.0 μM), those incubated with clonidine plus phentolamine were much less responsive ($P<0.01$).

It is well recognised that opiate agonists can suppress signs of withdrawal in opiate-dependent preparations. For example, normorphine restores to a normal level the tension of the contracture elicited by naloxone in isolated segments of guinea-pig ileum that have been incubated with normorphine (Collier *et al.*, 1980a). If the phentolamine-elicited contracture of the ileum indicated a state of dependence on clonidine, then application of additional clonidine immediately after challenge with phentolamine should restore the tension to a normal level. Figure 6a shows a typical tracing from one of six experiments in which the normally prolonged tension of contracture elicited by challenge with phentolamine (1.0 μM) was completely restored to resting level by application of clonidine (10 μM).

Site of clonidine dependence

That hexamethonium was always present in the Krebs solution eliminates the possibility that the clonidine dependence observed in this preparation occurred in a pre-ganglionic cholinergic neurone. To determine whether dependence occurred in a post-ganglionic neurone, we tested the effect of tetrodotoxin on the withdrawal-contracture. Figure 7 gives tracings from one of two experiments, in which tetrodotoxin completely abolished the response to phentolamine (1.0 μM) in ileal segments that had been incubated in clonidine (1.0 μM), and blocked responses to electrical stimulation in control and clonidine-incubated tissue. Tetrodotoxin did not affect responsiveness to challenge with ACh.

If the phentolamine-elicited contracture of clonidine-dependent ileum were due to the release of ACh from the final motor neurone, then it should be blocked by hyoscine, which blocks muscarinic receptors on the longitudinal muscle. Figure 6c which is a

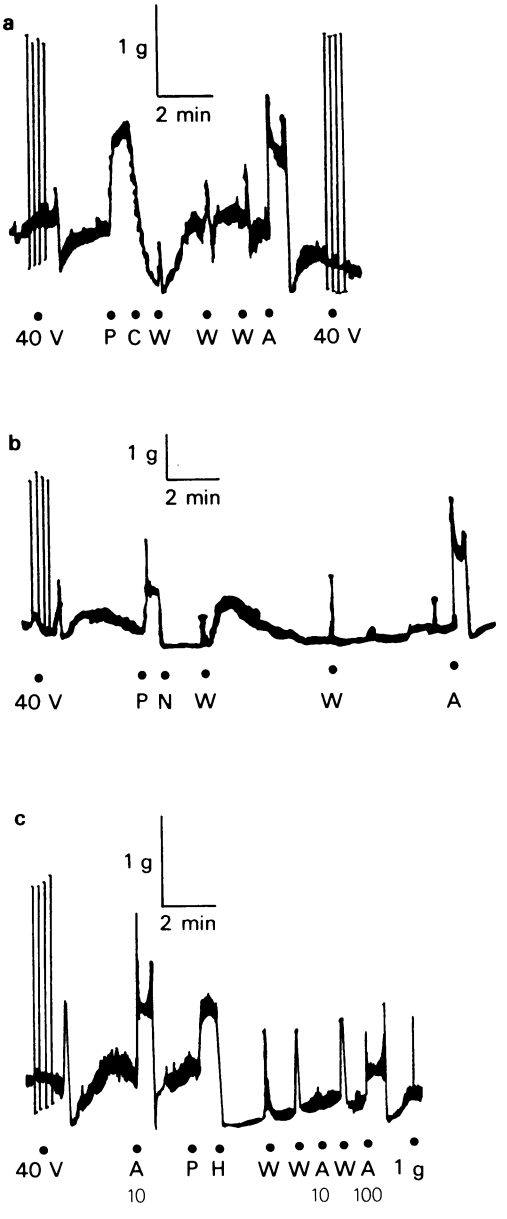

Figure 6 Effect of clonidine, normorphine or hyoscine on the contracture elicited by phentolamine challenge. Segments of ileum were incubated in fluid containing clonidine (1.0 μM). After equilibration for test at 37°C segments were challenged with phentolamine (1.0 μM) (P); 30–60s later, whilst the phentolamine-elicited contracture was at its peak, either (i) clonidine 10 μM (C, in a), (ii) normorphine 1.0 μM (N in b), or (iii) hyoscine 0.5 μM (H in c) was applied to the segments. After washing (W) segments were challenged with 10 nM ACh (A in a and b), or with 10 and 100 nM ACh (c). In (c) a reference response to 10 nM ACh was obtained before phentolamine challenge. Other details as in Figure 1.

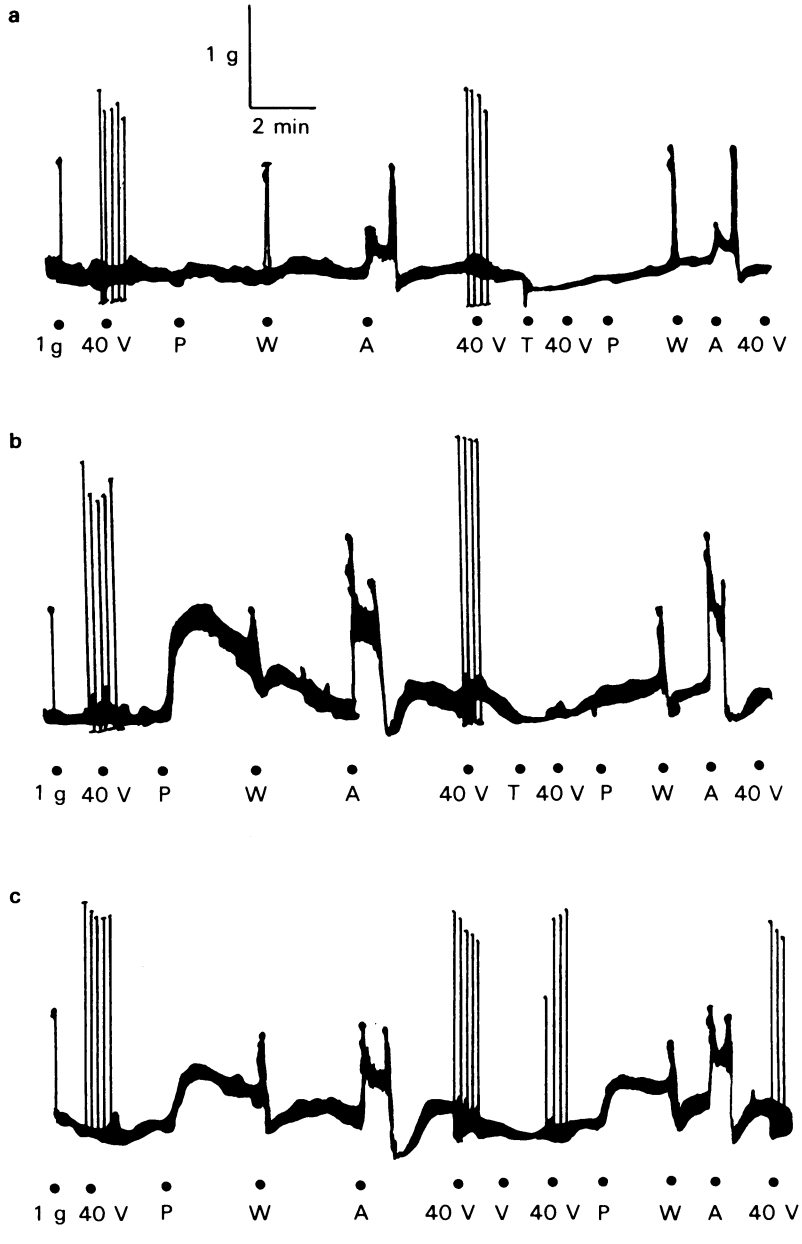

Figure 7 Effect of tetrodotoxin on the contracture elicited by phentolamine challenge. Segments of guinea-pig ileum, taken in threes from the same animal, were incubated in incubation fluid (a) or in this medium with the addition of clonidine 1.0 μM (b and c) and set up for test. After challenge with phentolamine 1.0 μM (P) and wash (W), each tissue was challenged with acetylcholine (ACh) 10 nM (A) and the 40 volt (40 V) stimulations repeated. After this, tetrodotoxin 3.0 μM (T) was applied to two of the segments (a and b) and the equivalent volume of distilled water (V) to the third (c). The testing sequence of 40 V stimulation followed by phentolamine 1.0 μM, ACh 10 nM and 40 V stimulation was then repeated. Other details as in Figure 1.

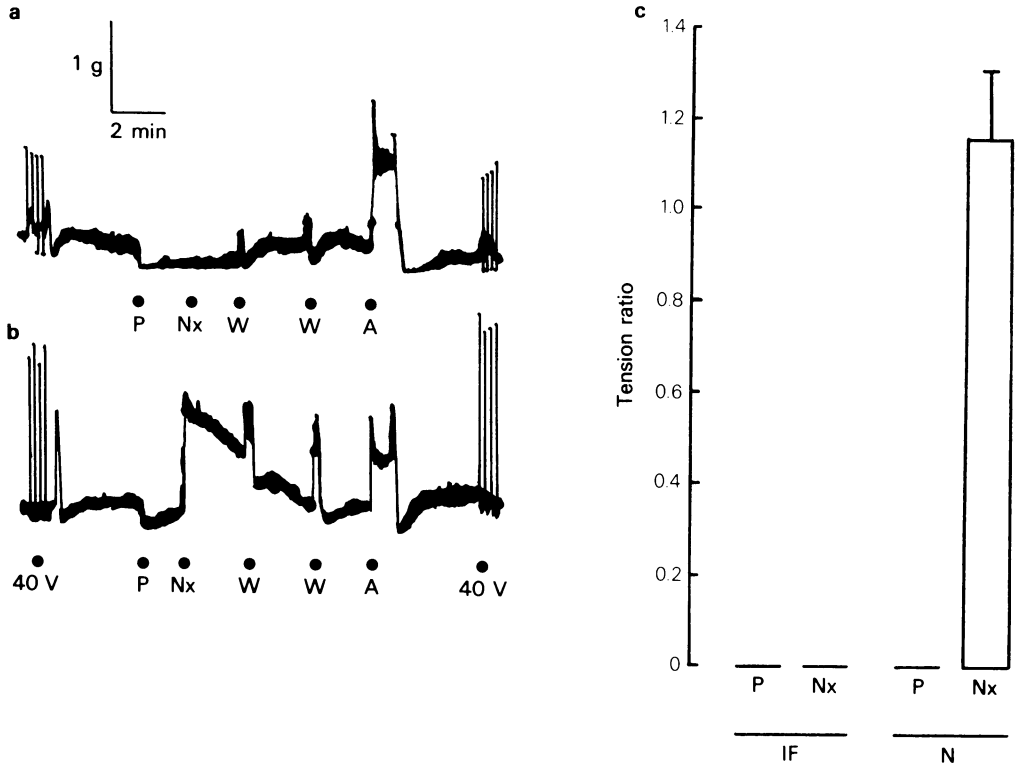

Figure 8 Effect of incubation in normorphine on sensitivity to phentolamine challenge. Segments of ileum, taken in pairs from the same animal, were incubated in incubation fluid alone (a and columns IF in c), or with the addition of normorphine 0.1 μM (b and columns N in c). After being incubated and set up for test, segments were challenged with phentolamine 1.0 μM (P), followed 1 min later by challenge with naloxone 0.1 μM (Nx). Columns (c) show the mean tension ratio and vertical bars the s.e.mean of 5 experiments. Other details as in Figure 1.

typical tracing from one of three experiments, shows that the tension of the contracture elicited by challenge with phentolamine (1.0 μM) in ileal segments incubated with clonidine (1.0 μM) was completely restored to the resting level by application of hyoscine (0.5 μM), which also reduced the response to subsequent application of ACh.

Relationship between clonidine- and normorphine-dependence

Figure 4 shows that naloxone (0.1 μM) did not elicit a contracture from preparations that had been incubated with clonidine (1.0 μM), although phentolamine (1.0 μM) was highly effective. Likewise, phentolamine (1.0 μM) did not elicit a contracture from segments previously incubated with normorphine (0.1 μM), although naloxone (0.1 μM) did so (Figure 8).

Figure 9 shows that clonidine (0.1 μM) readily suppressed the contracture elicited by naloxone (0.03 μM) in segments incubated with normorphine (1.0 μM) ($n = 6$). Conversely, normorphine (0.1 μM) suppressed the contracture elicited by phentolamine (1.0 μM) in segments incubated with clonidine (1.0 μM) (Figure 6b).

Thus, specific receptor antagonists elicited a contracture only from preparations incubated with the corresponding agonist, whereas specific agonists suppressed not only the withdrawal contracture of dependence on themselves, but also that of an inhibitory agonist acting on a different receptor.

Discussion

An important characteristic of drug dependence is that it does not occur immediately but takes appreciable time to be established. The induction by exposure for 24 h to clonidine of responsiveness to α-adrenoceptor antagonists fulfils this requirement, since no response to phentolamine occurred after incubation of the ileum for 0.5 h at 37°C with clonidine.

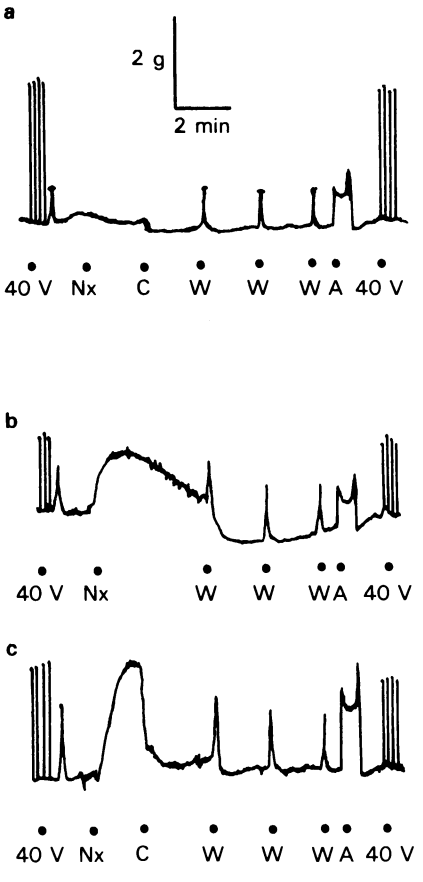

Figure 9 Effect of clonidine on the contracture elicited by naloxone in normorphine-dependent ileum. Segments of ileum, taken in threes from the same animal, were incubated in incubation fluid alone (a) or with the addition of normorphine 1.0 μM (b and c). After being incubated and set up for test in fluid equivalent to that used for incubation, segments were challenged with naloxone 30 nM (Nx). Two minutes later, when the contracture elicited by naloxone was at its peak, clonidine 0.1 μM (C) was applied (a and c). Other details as in Figure 1.

The above experiments indicate that incubation with clonidine induces in isolated segments of guinea-pig ileum a marked responsiveness to phentolamine, yohimbine and piperoxane, which specifically antagonize α-adrenoceptors situated presynaptically on the final cholinergic motor neurones of the myenteric plexus of the guinea-pig ileum (Kosterlitz & Watt, 1968; Deck *et al.*, 1971; Drew, 1978; Tanaka & Starke, 1979; Tayo, 1979). This phenomenon resembles the increase in responsiveness to naloxone that is induced by incubation of the guinea-pig ileum with opiate (Hammond *et al.*, 1976; Villarreal *et al.*, 1977; North & Karras, 1978; Collier *et al.*, 1980a,b). The increased responsiveness to α-adrenoceptor antagonists is specific in that (a) phentolamine inhibited its induction and (b) the β-adrenoceptor antagonist, propranolol, and the opiate antagonist, naloxone, did not elicit a contracture from ileum incubated with clonidine. We conclude from these and other properties and from a comparison between the phenomenon described in this paper and the more widely investigated opiate dependence in the ileum, that this is a model of clonidine dependence, in which the contracture elicited by α-adrenoceptor antagonists is a sign of withdrawal.

Clonidine dependence in the ileum differs, however, from the comparable opiate dependence in two respects. First, we have not yet detected any spontaneous contractions upon removal of clonidine from the incubation medium, although they are obvious after withdrawal of normorphine in the opiate-dependent ileum (Collier *et al.*, 1980a). Second, the increase in responsiveness to naloxone in the normorphine-dependent ileum is much greater than that to phentolamine in the clonidine-dependent preparation. These differences between clonidine and normorphine may perhaps be taken to mean that, in this model, clonidine has a lower capacity to induce dependence than has normorphine.

No examples could be found in the literature of withdrawal precipitated by an α-adrenoceptor antagonist in experimental animals chronically treated with an α-adrenoceptor agonist. However, cessation of chronic clonidine administration has been reported to induce disruption of behaviour in experimental animals (Meyer *et al.*, 1977) and a withdrawal syndrome in man (Reid *et al.*, 1977; Geyskes *et al.*, 1979). A marked increase in blood pressure and heart rate was seen after withdrawal in rats that had been treated with clonidine for periods of between 3 and 21 days (Oates, Stoker, Monaghan & Stokes, 1978). In the study by Oates *et al.*, no relationship could be found between the magnitude of the withdrawal response and the dose of clonidine given or the duration of exposure to clonidine; although the withdrawal response was reduced by clonidine and by a ganglionic blocker, indicating that it was neuronally mediated.

That hyoscine suppresses and tetrodotoxin blocks the clonidine withdrawal contracture, whereas hexamethonium prevents neither the induction nor the expression of dependence, indicates that the underlying change probably occurs in the final cholinergic motor neurone of the myenteric plexus. This cellular site corresponds with that proposed for opiate dependence in the ileum (Collier, 1980). That phentolamine does not elicit a normorphine withdrawal contracture and naloxone does not elicit a clonidine withdrawal contracture indicates that the receptors of this neurone involved in clonidine and in normorphine dependence are distinct, conforming with the

suggestion of Gillan *et al.* (1979). The receptors concerned presumably correspond with those for the acute actions of adrenergic and opiate agonists on this neurone, distinguished by Kosterlitz & Watt (1968). In contrast, the finding that normorphine suppresses the clonidine-withdrawal contracture and clonidine suppresses the normorphine-withdrawal contracture indicates that the withdrawal effects of both forms of dependence share a final common path, which is the release of ACh from this neurone. The interaction between clonidine and normorphine dependence in the final cholinergic motor neurone must therefore occur at a point between receptor binding and ACh release.

The observations summarized in the preceding paragraph appear to provide a simple experimental basis for the clinical finding that clonidine does not support opiate dependence, but relieves opiate withdrawal symptoms (Gold *et al.*, 1978, 1979; Washton *et al.*, 1979; Riordan & Kleber, 1980).

Although, after incubation in Krebs solution over 24 h, a slight response to phentolamine was sometimes obtained, in fresh preparations no contracture to phentolamine occurred. This can be conveniently explained by supposing that the myenteric plexus during incubation sometimes liberates enough noradrenaline to induce a slight dependence. We have not explored this possibility experimentally and other explanations are possible. A comparable 'self-induced dependence' has occasionally been noted in opiate dependence in the ileum (Collier *et al.*, 1980b).

We have previously developed a model of opiate dependence *in vitro*, based on the parallel incubation of segments of guinea-pig ileum in Krebs solution with or without added opiate. This has the advantages that, in a segment of ileum, dependence can be rapidly induced and readily measured in direct comparison with a control segment taken from the same animal (Collier *et al.*, 1980a,b). This model has now been extended to the study of clonidine dependence *in vitro*, and provides results that are consistent with what is known about clonidine dependence *in vivo*. These models can be used to elucidate the mechanisms of dependence on drugs of both types.

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References

AGHAJANIAN, G.K. (1978). Tolerance of locus coeruleus neurones to morphine and suppression of withdrawal response by clonidine. *Nature, Lond.*, **276**, 186–187.

COLLIER, H.O.J. (1980). Cellular site of opiate dependence. *Nature, Lond.*, **283**, 625–629.

COLLIER, H.O.J., CUTHBERT, N.J. & FRANCIS, D.L. (1980a). Effects of time and drug concentration on the induction of responsiveness to naloxone in guinea-pig ileum exposed to normorphine *in vitro*. *Br. J. Pharmac.*, **69**, 332P–333P.

COLLIER, H.O.J., CUTHBERT, N.J. & FRANCIS, D.L. (1980b). Tolerance, dependence and quasi-dependence in the guinea-pig isolated ileum. In *Endogenous and Exogenous Opiate Agonists and Antagonists*, ed. Way, E.L. pp.509–512. Oxford: Pergamon.

COLLIER, H.O.J., CUTHBERT, N.J. & FRANCIS, D.L. (1980c). Induction of responsiveness to phentolamine by incubation of guinea-pig ileum with clonidine *in vitro*. *Br. J. Pharmac.*, **70**, 175–176P.

CRAWLEY, J.N., LAVERTY, R. & ROTH, R.H. (1979). Clonidine reversal of increased norepinephrine metabolite levels during morphine withdrawal. *Eur. J. Pharmac.*, **57**, 247–250.

DECK, R. VON., OBERDORF, A. & KRONEBERG, G. (1971). The effect of 2-(2,b-dichlor-phenylamino-)-2-imidazoline-hydrochloride (Clonidine) on contraction of and acetylcholine liberation in the isolated guinea pig ileum stimulated coaxially-electrically. *Arzneimittelforsch.*, **21**, 1580–1584.

DREW, G.M. (1978). Pharmacological characterization of the presynaptic α-adrenoceptor regulating cholinergic activity in the guinea-pig ileum. *Br. J. Pharmac.*, **64**, 293–300.

EHRENPREIS, S., LIGHT, I. & SCHONBUCH, G.H. (1972). Use of the electrically stimulated guinea-pig ileum to study potent analgesics. In *Drug Addiction: Experimental Pharmacology*, ed. Singh, J.M., Miller, L.H. & Lal, H. pp.319–342. New York: Futura.

FIELDING, S., WILKER, J., HYNES, M., SZEWCZAK, M., NOVICK, W.J. & LAL, H. (1978). A comparison of clonidine with morphine for antinociceptive and anti-withdrawal actions. *J. Pharmac. exp. Ther.*, **207**, 899–905.

GEYSKES, G.G., BOER, P. & DORHOUT-MEES, E.J. (1979). Clonidine withdrawal mechanism and frequency of rebound hypertension. *Br. J. clin. Pharmac.*, **7**, 55–62.

GILLAN, M.G.C., KOSTERLITZ, H.W., ROBSON, L.E. & WATERFIELD, A.A. (1979). The inhibitory effects of presynaptic α-adrenoceptor agonists on contractions of guinea-pig ileum and mouse vas deferens in the morphine-dependent and withdrawn states produced *in vitro*. *Br. J. Pharmac.*, **66**, 601–608.

GOLD, M.S., POTTASH, A.L.C., SWEENEY, D.R. & KLEBER, H.D. (1979). Clonidine detoxification: a fourteen-day protocol for rapid opiate withdrawal. In *Problems of Drug Dependence 1979*, ed. Harris, L.S. pp. 226–232. Washington D.C.: National Institute on Drug Abuse.

GOLD, M.S., REDMOND, D.E.Jr. & KLEBER, H.D. (1978). Clonidine in opiate withdrawal. *Lancet*, **i**, 929–930.

GYANG, E.A. & KOSTERLITZ, H.W. (1966). Agonist and

antagonist actions of morphine-like drugs on the guinea-pig isolated ileum. *Br. J. Pharmac. Chemother.*, **27**, 514–527.

HAMMOND, M.D., SCHNEIDER, C. & COLLIER, H.O.J. (1976). Induction of opiate tolerance in isolated guinea pig ileum and its modification by drugs. In *Opiates and Endogenous Opioid Peptides*, ed. Kosterlitz, H.W. pp.169–176. Amsterdam: Elsevier/North-Holland Biomedical Press.

HANSSON, L., HUNYOR, S.N., JULIUS, S. & HOOBLER, S.W. (1973). Blood pressure crisis following withdrawal of clonidine (Catapres, Catapreson), with special reference to arterial and urinary catecholamine levels, and suggestions for acute management. *Am. Health J.*, **85**, 605–610.

JENNEWEIN, H.M., STOCKHAUS, K. & HOEFKE, W. (1980). Effects of clonidine and elinidine in the morphine withdrawal syndrome in rats and dogs. *Naunyn-Schmiedebergs Arch. Pharmac.*, **311**, Suppl. R66 (Abstract No.263).

KOSTERLITZ, H.W. & WATT, A.J. (1968). Kinetic parameters of narcotic agonists and antagonists, with particular reference to N-allylnoroxymorphone (naloxone). *Br. J. Pharmac.*, **33**, 266–276.

KRONEBERG, G. & OBERDORF, A. (1971). Inhibition of acetylcholine release and acetylcholine action in the guinea pig ileum by sympathetic α- and β-receptor stimulation. *First Congress of the Hungarian Pharmacological Society, Budapest*, **1**, 39–48.

LAVERTY, R. & ROTH, R.H. (1980). Clonidine reverses the increased norepinephrine turnover during morphine withdrawal in rats. *Brain Res.*, **182**, 482–485.

MEYER, D.R., EL-AZHARY, R., BIERER, D.Ws., HANSON, S.K., ROBBINS, M.S. & SPARBER, S.B. (1977). Tolerance and dependence after chronic administration of clonidine to the rat. *Pharmac. Biochem. Behav.*, **7**, 227–231.

NORTH, R.A. & KARRAS, P.J. (1978). Opiate tolerance and dependence induced *in vitro* in single myenteric neurones. *Nature, Lond.*, **272**, 73–75.

OATES, H.F., STOKER, L.M., MONAGHAN, J.C. & STOKES, G.S. (1978). Withdrawal of clonidine: Effects of varying dosage or duration of treatment on subsequent blood pressure and heart rate responses. *J. Pharmac. exp. Ther.*, **206**, 268–273.

REID, J.L., DARGIE, H.J., DAVIES, D.J., WING, L.M.H., HAMILTON, C.A. & DOLLERY, C.T. (1977). Clonidine withdrawal in hypertension. *Lancet*, **i**, 1171–1174.

RIORDAN, C.E. & KLEBER, H.D. (1980). Rapid opiate detoxification with clonidine and naloxone. *Lancet*, **i**, 1079–1080.

SABOL, S.L. & NIRENBERG, M. (1979). Regulation of adenylate cyclase of neuroblastoma × glioma hybrid cells by α-adrenergic receptors. *J. biol. Chem.*, **254**, 1921–1926.

SPARBER, S.B. & MEYER, D.R. (1978). Clonidine antagonizes naloxone-induced suppression of conditioned behaviour and body weight loss in morphine-dependent rats. *Pharmac. Biochem. Behav.*, **9**, 319–325.

TANAKA, T. & STARKE, K. (1979). Binding of ^{3}H-clonidine to an α-adrenoceptor in membranes of guinea-pig ileum. *Naunyn-Schmiedebergs Arch. Pharmac.*, **309**, 207–215.

TAYO, F.M. (1979). Prejunctional inhibitory α-adrenoceptors and dopaminoceptors of the rat vas deferens and the guinea-pig ileum *in vitro*. *Eur. J. Pharmac.*, **58**, 189–195.

TSENG, L-F, LOH, H.H. & WEI, E.T. (1975). Effects of clonidine on morphine withdrawal signs in the rat. *Eur. J. Pharmac.*, **30**, 93–99.

VETULANI, J. & BEDNARCZYK, B. (1977). Depression by clonidine of shaking behaviour elicited by nalorphine in morphine-dependent rats. *J. Pharm. Pharmac.*, **29**, 567–569.

VILLARREAL, J.E., MARTINEZ, J.N. & CASTRO, A. (1977). Validation of a new procedure to study narcotic dependence in the isolated guinea-pig ileum. In *Problems of Drug Dependence, 1977*, pp.305–314. Washington D.C.: Committee on Problems of Drug Dependence Inc.

WASHTON, A.M., RESNICK, R.B. & RAWSON, R.A. (1979). Clonidine hydrochloride: a nonopiate treatment for opiate withdrawal. In *Problems of Drug Dependence 1979*, ed. Harris, L.S. pp.233–239. Washington D.C.: National Institute on Drug Abuse.

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