Effects of morphine and chlorpromazine on apomorphine-induced stereotyped behaviour

Recent studies have suggested that morphine may block dopamine receptors in the striatum. An elevated homovanillic acid content (Kuschinsky & Hornykiewicz, 1972) and an increased rate of conversion of [^{3}H]tyrosine to [^{3}H]dopamine (Gauchy, Agid & others, 1973) have been demonstrated in striatal tissue of morphine-treated rats. Puri, Reddy & Lal (1973) have reported that morphine antagonized apomorphine-induced stereotyped behaviour and accelerated the depletion of rat striatal dopamine following α-methyltyrosine treatment. These biochemical and behavioural effects are characteristic of agents, such as chlorpromazine and haloperidol, which are thought to be dopamine receptor blockers.

In our laboratory morphine has not proved to be an effective antagonist of apomorphine-induced stereotyped behaviour. To demonstrate this we have compared the effects of morphine sulphate and chlorpromazine pretreatments on apomorphine-induced stereotyped behaviour.

Sprague Dawley male rats, 200–250 g, were placed in circular wire mesh cages until all exploratory activity had ceased. Animals were pretreated intraperitoneally with either saline or test drug 30 min before apomorphine was given subcutaneously. Following apomorphine, animals were scored for intensity of stereotyped behaviour for 1 h using the scoring system described by McKenzie (1971). Test compounds were prepared in saline whereas apomorphine was dissolved in 0·001 N HCl.

Chlorpromazine, 2–30 mg kg^{-1} (i.p.), caused a marked reduction in mean behavioural scores (Table 1). Stereotyped behaviour induced by 1 mg kg^{-1} of apomorphine was reduced from a mean score of 9·0 ± 0·6 to 4·5 ± 0·5 following 2 mg kg^{-1} of chlorpromazine. Higher doses of chlorpromazine completely blocked the stereotypy. When the dose of apomorphine was increased by factors of 5 and 10, the dose of chlorpromazine required to reduce mean scores by 50% was increased by factors of approximately 5 and 10, respectively.

In contrast to chlorpromazine, morphine, 0·2–40 mg kg^{-1}, did not block stereotyped behaviour (Table 2). Morphine pretreatment, 10–40 mg kg^{-1}, consistently increased mean behavioural scores. This effect was most marked in animals treated with 1 mg kg^{-1} of apomorphine where mean stereotypy scores increased from a control value

Table 1. *Antagonism of apomorphine-induced stereotyped behaviour by chlorpromazine.*

Chlorpromazine mg kg^{-1}	Mean scores ± s.e. after apomorphine: 1 mg kg^{-1}	5 mg kg^{-1}	10 mg kg^{-1}
—	9·0 ± 0·6 (26)	18·8 ± 1·0 (20)	19·4 ± 0·8 (28)
1	6·0 ± 0·5** (6)	14·6 ± 2·2 (6)	20·2 ± 1·9 (10)
2	4·5 ± 0·5*** (6)	15·1 ± 2·6 (6)	19·5 ± 1·5 (10)
5	1·8 ± 0·4*** (6)	15·8 ± 1·8 (6)	19·0 ± 2·0 (10)
10	0·5 ± 0·2*** (6)	8·6 ± 2·3** (6)	16·1 ± 2·0 (10)
20	0 *** (2)	6·5 ± 1·5*** (6)	12·8 ± 1·0*** (6)
30	0·5 ± 0·5*** (2)	3·0 ± 0·3*** (6)	15·8 ± 1·5* (6)

Chlorpromazine was administered i.p., 30 min before apomorphine s.c. Animals were scored for 1 h following apomorphine. *$P<0·05$, **$P<0·01$, ***$P<0·001$ compared to the appropriate control dose of apomorphine. P values were calculated using Student's t-test. (N) = number of animals.

Table 2. *Effects of morphine on apomorphine-induced stereotyped behaviour.*

Morphine mg kg^{-1}	Mean scores ± s.e. after apomorphine: 1 mg kg^{-1}	5 mg kg^{-1}	10 mg kg^{-1}
—	9·0 ± 0·6 (26)	18·8 ± 1·0 (20)	19·4 ± 0·8 (28)
0·2	7·9 ± 0·5 (7)	19·7 ± 1·0 (6)	21·6 ± 1·4 (8)
0·5	7·7 ± 0·4 (7)	19·5 ± 2·0 (6)	22·4 ± 0·8*
2·0	9·0 ± 0·6 (16)	20·2 ± 0·9 (12)	19·7 ± 1·2 (12)
5·0	10·6 ± 0·7 (10)	21·1 ± 0·7 (12)	22·3 ± 1·2 (8)
10	12·0 ± 1·6 (8)	22·1 ± 0·7*** (14)	23·3 ± 0·9** (8)
20	17·8 ± 0·8*** (14)	22·8 ± 0·8** (16)	23·8 ± 0·8** (16)
40	17·9 ± 1·3*** (8)	23·3 ± 0·8** (8)	19·8 ± 2·1 (16)

Morphine sulphate administered i.p., 30 min before apomorphine s.c. Animals were scored for 1 h following apomorphine. *$P<0·05$, **$P<0·01$, ***$P<0·001$ compared to the appropriate control dose of apomorphine. P values were calculated using Student's t-test. (N) = number of animals.

of 9·0 ± 0·6 to 17·9 ± 1·3 ($P<0·001$) following 40 mg kg^{-1} of morphine. At the higher doses of apomorphine, potentiation by high doses of morphine, though less pronounced, was present and in most cases statistically significant.

Morphine treatment alone did not produce stereotyped behaviour.

The antagonistic activity of chlorpromazine is consistent with the results of other studies demonstrating that apomorphine-induced stereotyped behaviour can be effectively antagonized by antipsychotic agents (Dhawan, Saxena & Gupta, 1961; Ernst, 1969; Rotrosen, Wallach & others, 1972; McKenzie, Viik & Boyer, 1973). Furthermore, the present findings suggest that the interaction between chlorpromazine and apomorphine is competitive, presumably at dopaminergic receptor sites. Lahti, McAllister & Wozniak (1972) arrived at a similar conclusion using biochemical criteria. These results are consistent with the concept that chlorpromazine blocks dopamine receptors in the central nervous system (Carlsson & Lindqvist, 1963; van Rossum, 1966).

Our results are in direct contrast to those of Puri & others (1973). In our hands, morphine produced potentiation rather than blockade of stereotyped behaviour, an observation consistent with the report that morphine increased the frequency of occurrence and duration of apomorphine stereotypy (Vedernikov, 1970).

It is concluded that morphine does not effectively antagonize, but rather potentiates, apomorphine-induced stereotyped behaviour especially at low doses of apomorphine. Furthermore, if morphine has a direct effect on dopaminergic neurons and/or receptors as indicated by changes in the metabolic disposition of dopamine (Puri & others, 1973; Kuschinsky & Hornykiewicz, 1972), it is probably by some mechanism different than that of chlorpromazine. A similar conclusion has been drawn recently by Kuschinsky & Hornykiewicz (1972).

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A refined procedure for determining pA_2 values

pA_2 Values (Schild, 1947; Arunlakshana & Schild, 1959) are widely used to quantify the affinity of antagonists for a receptor site and also to characterise receptor types *in vitro*. Recently, this method has been used to measure "apparent" pA_2 values *in vivo* (Hayashi & Takemori, 1971). The method is based on a plot of $\log_{10}$ (dose ratio -1) against $-\log_{10}$ molar concentration of antagonist. However, this procedure does not make the best use of all the available information and may lead to inaccuracies in the estimation of pA_2. We present a new procedure for measuring pA_2 values that does not possess these disadvantages.

The method of Schild is to estimate the parameters α (intercept on the ordinate) and β (slope) of the straight line:—

$$\log_{10}\left(\frac{C_m}{C_0}-1\right) = \alpha + \beta \log_{10} M \quad .. \quad .. \quad .. \quad (1)$$

where C_0 is the dose (mg litre^{-1} *in vitro* or mg kg^{-1} *in vivo*) of the agonist alone which causes 50% of the response being measured (ED50 value) and C_m is the ED50 value of the agonist in the presence of M mol litre^{-1} *in vitro* or M mol kg^{-1} *in vivo* of antagonist. Thence pA_2 is given by:—

$$pA_2 = \frac{\alpha}{\beta} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

Although equation (1) is linear and therefore amenable to elementary treatment, the direct relationship between the potency of the agonist (C_m) and the molar concentration of the antagonist (M) is not linear. The direct relationship which is obtained by rearrangement of equation (1) is:—

$$C_m = C_0'(1 + 10^{\alpha} M^{\beta}) \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where all the symbols have the same meaning as in equation (1) except that C_0' is the estimate of the ED50 value for the agonist alone. The pA_2 is derived from equation (2) as before. The advantages of using equation (3) to determine pA_2 values instead of equation (1) are illustrated by reference to Fig. 1.