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Antagonism by physostigmine of the "running fit" caused by levorphanol, a morphine congener, in mice

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1. Drugs of the morphine type cause a stereotyped " running fit " in the mouse.

2. The intensity and duration of this response are related to the dose.

3. Measurement of this phenomenon serves as a good method for the quantitative comparison of drugs of this type and for the study of their antagonists.

4. Intracerebral injection of physostigmine antagonized the " running fit " induced by a wide range of doses of levorphanol.

5. The results are consistent with the hypothesis that drugs of the morphine type act by retarding the release of acetylcholine at some central cholinergic synapses.

Paton (1957) showed that contractions of the transmurally stimulated guinea-pig ileum could be depressed by morphine and related analgesic drugs, and this effect was attributed to inhibition of acetylcholine release. Schaumann (1957) proposed that central actions of morphine and its congeners might also result from inhibition of acetylcholine release at cholinergic synapses. Cox & Weinstock (1966) found a close relationship between the structure-activity requirements for analgesic effect and those for inhibition of contraction of the guinea-pig ileum. Morphine was shown to diminish the amount of acetylcholine released into perfused lateral ventricle and subarachnoid space of the cat (Beleslin, Polak & Sproull, 1965; Beleslin & Polak, 1965). And, recently, it was demonstrated that morphine reduces the amount of readily extractable (" free ") acetylcholine in rat brain; whereas physostigmine acts in an opposite way, presumably because of its well known inhibitory action on brain acetylcholinesterase (Crossland & Slater, 1968). Thus several lines of evidence suggest that certain effects of morphine and its congeners on the central nervous system may be mediated by blockade of acetylcholine release from nerve terminals. If that is true, physostigmine, which protects released acetylcholine from destruction, ought to antagonize such effects of morphine and

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related drugs. Shaw & Bentley (1952) provided some evidence that physostigmine can arouse dogs that have been depressed severely by morphine.

Current research in this laboratory is concerned with tolerance resulting from administration of levorphanol, a synthetic congener of morphine, in mice. An exceedingly useful method of quantifying the tolerance phenomenon employs, as criterion of drug action, the " running fit," the intensity and duration of which can be measured automatically in individual mice or groups of mice by means of cages equipped with photoelectric counters. This characteristic activity is evoked by morphine and its analgesically active congeners in the D(−) conformation but not by the L(+) isomers ; and it is promptly abolished by nalorphine. The intensity and duration of the stereotyped running activity are dose-related, and tolerance can be produced readily and predictably by appropriate choice of dosage and frequency of administration (Goldstein & Sheehan, 1969).

If the " running fit " were a consequence of diminished acetylcholine release at some specific brain site, it would be expected that physostigmine should antagonize the levorphanol-induced running activity throughout a wide range of levorphanol dosage. The purpose of the experiments described was to examine the validity of this prediction.

Methods

Male Swiss-Webster mice (20–30 g) were placed, in groups of three, in rectangular cages (13 × 28 cm, 13 cm high) with wire-screen floors. The running activity was measured using modifications of the methods introduced by Winter & Flataker (1951) and Dews (1953). A beam of light was passed across the short axis of the cage midway between the two ends ; whenever a mouse interrupted this beam a cumulative counter was advanced. Thus a continuous record was made of running activity against elapsed time. After a period of adaptation to the cage only a few counts per hour are recorded for uninjected mice or mice that have received an injection of normal saline solution or sodium tartrate subcutaneously. Levorphanol tartrate at a maximally effective dose (approximately 12–20 mg/kg of the free base) produces several thousand counts during the period of 2–3 hr after drug administration.

The mice were allowed free access to food and water until they were placed in the experimental cages in a quiet room. After 30–45 min they were given either levorphanol or normal saline solution subcutaneously, in a volume no greater than 0·3 ml. After another 15 min physostigmine sulphate (1 μg of the free base) in saline solution or saline solution alone was injected (10 μl.) into a lateral ventricle of the brain, as described by Haley & McCormick (1957). The running activity was then recorded for 1 hr in most experiments. The running activity was recorded for 3 hr in experiments presented in Fig. 1. The data are presented as mean cumulative counts (with standard errors) for five cages (three mice in each) at each levorphanol dose. The dose-response data in Fig. 2 consisted of four doses in the saline treated group, five doses in the physostigmine treated group. All the data from the forty-five cages were analysed according to Finney (1964).

Levorphanol tartrate was generously donated by Hoffman-LaRoche, Inc. Physostigmine sulphate was purchased from Merck & Co. All doses are expressed in terms of free base.

Results

Figure 1 shows the running activity of mice over a period of 3 hr following a dose of 5·8 mg/kg of levorphanol administered subcutaneously with either saline or physostigmine given intracerebrally. In the saline treated group, the curve is linear for about 90 min, then flattens rather abruptly after 2 hr. Following intracerebral injection of physostigmine, the levorphanol-induced running activity was greatly reduced for about 20 min ; subsequently, the running rate increased. After about 2 hr the curve flattened as did that of the saline treated group.

Physostigmine alone, given intracerebrally to mice that had not received levorphanol, produced nothing remarkable other than a moderate reduction of spontaneous activity after the initial stunning effect of the injection. Similar injection of saline solution alone caused momentary stunning, but after a few minutes, the animals' behaviour appeared quite normal again.

Figure 2 presents the results of all the dose-response experiments, covering a range of levorphanol dosage from 1·45 to 11·6 mg/kg in the saline controls and from 2·90 to 46·4 mg/kg in the animals receiving physostigmine intracerebrally.

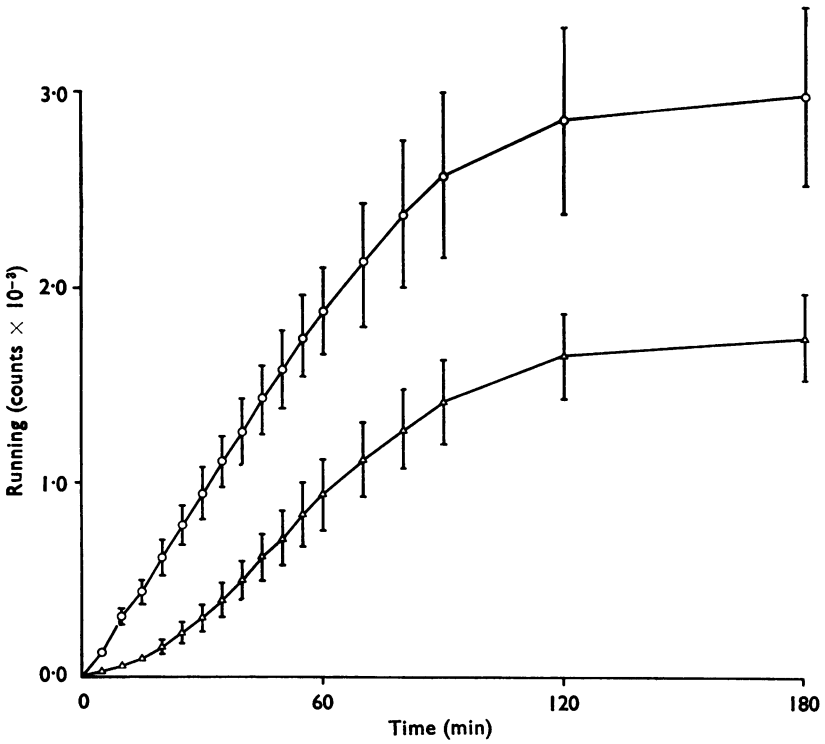

FIG. 1. Time course of running activity induced by levorphanol in mice. Each point represents the mean cumulative running activity (± standard error) for five cages containing three mice each, for a period of 3 hr after intraventricular injection of saline solution (10 μl.) or physostigmine (1 μg in 10 μl. saline solution). All mice had been given levorphanol tartrate 5·8 mg/kg (calculated as free base) 15 min earlier. ○——○, Levorphanol-saline group; △——△, levorphanol-physostigmine group.

The analysis of variance for the same data is given in Table 1. The highly significant variance ratios for regression and for treatments substantiate the validity of the assay and show a real effect of physostigmine. The low variance ratio for deviation from linearity further validates the use of linear regression equations. Finally, the low variance ratio for deviation from parallelism indicates that physostigmine did not significantly alter the slope of the log dose-response line. The

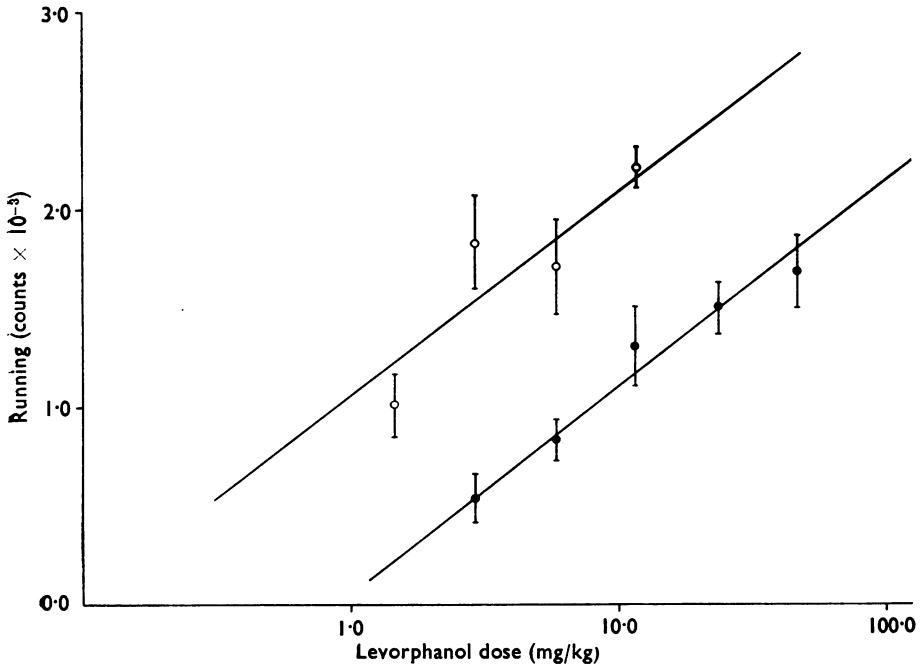

FIG. 2. Antagonism by physostigmine of running activity induced by levorphanol in mice. Each point represents the mean cumulative running activity (± standard error) for five cages containing three mice each, for a period of 1 hr after intraventricular injection of saline solution (10 μl.) or physostigmine (1 μg in 10 μl.). All mice had been given levorphanol tartrate at the indicated dose (of free base) 15 min earlier. Maximum running activity under these conditions is approximately 2,200 counts in 1 hr at a dose of 12–20 mg/kg in the absence of physostigmine. The parallel regression lines are drawn to the regression equations computed from the raw data, using the pooled slope estimate; this slope is 1,042 if the units of the abscissae are $\log_{10}$ (dose). ○, Saline; ●, physostigmine.

TABLE 1. *Analysis of variance*

Components	*DF*	*SS* (× 10^{-6})	Variance estimate (× 10^{-6})	*F*
Treatments	1	3·009	3·009	20·2*
Regression	1	7·376	7·376	49·5*
Deviation from parallelism	1	0·043	0·043	0·3
Deviation from linearity	5	0·888	0·178	1·2
Between doses	8	11·316	1·420	
Error	36	5·357	0·149	
Total	44	16·673	0·379	

For the computations, the doses were converted to $\log_2$ and coded so that at dose 5·80 mg/kg, $x=0$. There were five cages of three mice at each dose, four doses in the saline group, five doses in the physostigmine group. In the column at extreme right are shown ratios of each variance estimate to the error variance. * Significantly greater than unity, $P<0·01$.

effect of physostigmine was to shift this line to the right by 0·951 $\log_{10}$ units of dose. The computed potency ratio (levorphanol dose with physostigmine/levorphanol dose with saline) is 8·9, with 99% confidence limits 3·8 to 20·9. Thus, within the range tested, physostigmine increased by a factor of about 9 the levorphanol dose required to elicit any given intensity of running activity.

Discussion

The observation in Fig. 1 that the antagonistic action of physostigmine was greatest for about the first 20 min and then diminished gradually could probably be due to a concomitant removal of physostigmine from the ventricles and the brain tissue by circulation of the cerebrospinal fluid. Comparably rapid disappearance of radioactive inulin and other substances from the mouse brain after intraventricular injections has been demonstrated in this laboratory by S. N. Giri (unpublished observations). It would seem likely then, that the amount of physostigmine used in the dose-response experiments would have shown, at every levorphanol dose, greater antagonistic effect than observed, had it remained in the brain for the entire 1 hr period of measurement.

Recent experiments carried out in this laboratory showed that the depletion of noradrenaline, but not 5-hydroxytryptamine, prevents levorphanol-induced running fits in mice (M. Hollinger, unpublished observations). This suggests that the diminution of acetylcholine may not be the only factor in the production of running fits in mice and that this phenomenon may normally be modulated by a dual antagonist innervation, cholinergic inhibiting and adrenergic stimulating.

Nevertheless, our results are unequivocal in showing that the " running fit " caused by levorphanol in mice is antagonized by physostigmine. The route of administration of physostigmine, the small dose (1 μg), and the time course of the effect indicate that this antagonism, like the levorphanol action itself, is localized in the brain. These findings are also in agreement with the idea that the " running fit " may be caused by diminution of acetylcholine at some synaptic site. Conclusive proof will have to await direct demonstration that levorphanol diminishes acetylcholine release (and that physostigmine, which protects released acetylcholine from destruction, overcomes this diminution) at a brain site associated with the initiation of running activity.

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