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THE MODIFICATION BY PHYSOSTIGMINE OF SOME EFFECTS OF NICOTINE ON BAR-PRESSING BEHAVIOUR OF RATS

BY

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Physostigmine has been shown to potentiate the twitching of the ears and the fall in blood pressure which occur when nicotine is injected into a lateral ventricle of an anaesthetized cat (Armitage, Milton & Morrison, 1966 ; Armitage & Hall, 1967). Both these effects of nicotine result from a direct action at two different sites within the central nervous system. It was therefore of interest to see if physostigmine modified any behavioural effects of nicotine which are presumed to be central in origin. The effects of nicotine on the behaviour of thirsty rats trained to press a bar in order to get a water reward have already been described (Morrison, 1967). When bar-pressing responses are rewarded at varying intervals the rats press the bar at a steady rate and this type of performance is particularly sensitive to the effects of nicotine. A variable-interval schedule of reward with an average interval of 2 min between rewards was therefore chosen to study the behavioural interactions of nicotine and physostigmine.

METHODS

Operant behaviour

The animals used were four male Lister black-hooded rats which were 200–220 days old at the start of the experiment and weighed between 234 and 292 g.

The rats were deprived of water for 21 hr/day and were then trained in Grason Stadler Skinner boxes to press a bar for water rewards. During the first two training sessions they received 0.1 ml. water each time they pressed the bar ; they were then put directly onto a variable-interval schedule in which bar-pressing responses were rewarded at varying intervals, the average time between rewards being 2 min. Each rat was tested for 90 min daily from Monday to Friday and had free access to water for a further 90 min after each experimental session and from 5 p.m. on Friday until noon on Sunday. The experiment was started after 7 days' training on the variable-interval schedule.

Saline was injected subcutaneously immediately before the start of the sessions on Tuesdays and Thursdays and drugs were administered on Wednesdays and Fridays. Nicotine, physostigmine and neostigmine were injected subcutaneously immediately before the start of the experimental sessions when tested alone. In the drug interaction studies the anticholinesterase was administered 10 min before the nicotine.

Each rat received four doses of nicotine (0.05, 0.1, 0.2 and 0.4 mg/kg) which were tested with and without pretreatment with physostigmine 0.05 mg/kg. In addition physostigmine 0.05 mg/kg was tested alone on four occasions. This dose of physostigmine was selected on the basis of a

preliminary experiment in which it was found to cause only a slight (9%) depressant effect on variable-interval performance. The highest dose of nicotine (0.4 mg/kg) was also tested after pretreatment with neostigmine 0.05 mg/kg, which was tested alone on one occasion. The reason for using a dose of neostigmine similar to that of physostigmine is discussed later in the paper.

The experiments were divided into groups of three so that each dose of nicotine, the same dose after pretreatment with anticholinesterase and the anticholinesterase alone were administered on consecutive test days. Thus for each dose of nicotine a comparison could be made among three treatments which had been carried out within the space of a week. The four doses of nicotine were administered in ascending order, each rat starting with a different dose, and the dose of 0.05 mg/kg following that of 0.4 mg/kg.

Nicotine hydrogen tartrate, physostigmine sulphate and neostigmine bromide were dissolved in 0.9% saline to give an injection volume of 0.1 ml./100 g body weight. All doses are expressed as base.

An analysis of variance was carried out at each dose level of nicotine to investigate differences among (*a*) drug treatments, (*b*) rats, and (*c*) size of drug effect during successive 10-min intervals. The analysis was based on the absolute differences between scores for drug and control experiments.

RESULTS

Fig. 1 shows the effects on bar-pressing performance of nicotine 0.2 mg/kg, physostigmine 0.05 mg/kg and of the two drugs together. The number of times the bar was pressed was measured separately every 10 min. The nine scores for each experimental session were compared with the corresponding scores for the preceding control day. The difference was expressed as a percentage of the control score. Nicotine caused an increase in bar-pressing rate whereas physostigmine had little effect. When the rats had been pretreated with physostigmine, nicotine had little effect on response rate. The lower doses of nicotine (0.05 and 0.1 mg/kg) also increased the rate of bar-pressing and this

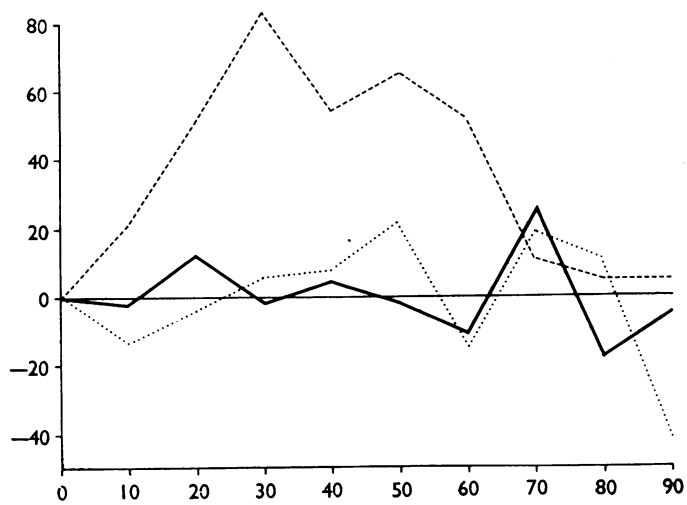

Fig. 1. Effects of nicotine, physostigmine and of the two drugs together on the performance of rats pressing a bar for water on a variable-interval schedule of reward. Ordinate: per cent change in the total number of responses during each 10 min period, the difference between experiment and control scores being expressed as a percentage of the control. Abscissa: time in minutes from the start of the session. - - - -: Nicotine 0.2 mg/kg; · · · ·: physostigmine 0.05 mg/kg; ——: physostigmine followed by nicotine.

effect was again abolished by pretreatment with physostigmine. Analysis of variance showed that at all these dose levels the nicotine effect differed significantly ($P<0.01$) from the effects of either physostigmine or of physostigmine and nicotine, but that the latter treatments did not differ from each other.

At the highest dose (0.4 mg/kg) the effect of nicotine again differed significantly ($P<0.01$) from either of the other treatments (Fig. 2). The drug effect was not uniform

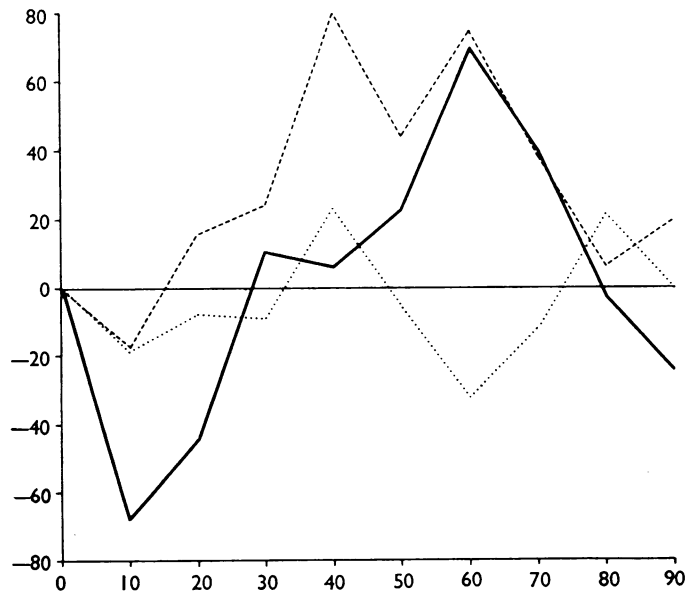

Fig. 2. Details as in Fig. 1. - - - -: Nicotine 0.4 mg/kg; · · · ·: physostigmine 0.05 mg/kg; ——: physostigmine followed by nicotine.

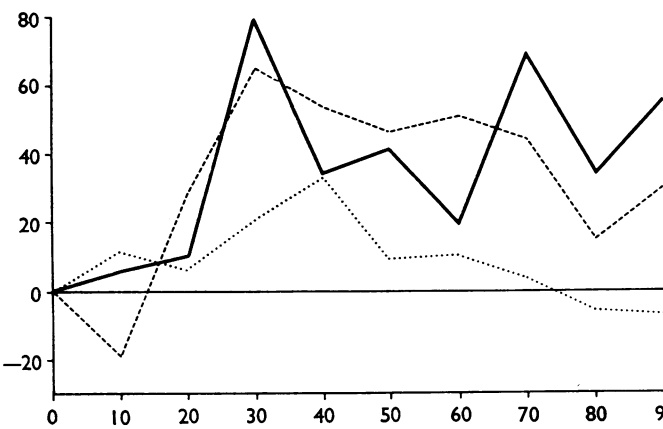

Fig. 3. Effects of nicotine, neostigmine and of the two drugs together on the performance of rats pressing a bar for water on a variable-interval schedule of reward. Ordinate and abscissa as in Fig. 1. - - - -: Nicotine 0.4 mg/kg; · · · ·: neostigmine 0.05 mg/kg; ——: neostigmine followed by nicotine.

for the successive 10 min time intervals. Nicotine caused an initial reduction in the rate of bar-pressing which was followed by a phase of increased response rate. This reduction was significantly ($P<0.01$) potentiated by pretreatment with physostigmine while the subsequent increase in rate was reduced but not abolished.

An analysis of variance was also carried out on the results obtained with neostigmine. The effect of neostigmine was significantly ($P<0.01$) different from that of nicotine but pretreatment with neostigmine did not alter the effect of the nicotine (Fig. 3).

DISCUSSION

The highest dose of nicotine (0.4 mg/kg) caused a brief initial reduction in bar-pressing to a rate which did not differ significantly from the controls. In a previous study (Morrison, 1967) this dose of nicotine was found to reduce significantly the initial rate of bar-pressing of a larger group of rats working on the same schedule. There is considerable variation in the extent to which individual rats are affected by this dose and the four rats used in the present study were particularly unsusceptible to the depressant phase of the nicotine effect. The data from the previous study indicate that although the depression was not in this case statistically significant the effect was nevertheless genuine.

The results are presented as a comparison of response rates at different times during drug and control sessions. It is assumed that at any time differences in response rate are caused by an action of the drug and not by impending satiation, because the nature of the schedule is such that there is very little difference in the number of rewards received by a slowly and a rapidly responding animal. This point has been discussed more fully in a previous paper (Morrison, 1967).

It is a reasonable assumption that the effects of physostigmine 0.05 mg/kg described in this paper are caused by inhibition of cholinesterase, because this is a minimally effective dose of a substance which is classified pharmacologically as a cholinesterase inhibitor.

If the site of interaction of nicotine and physostigmine were peripheral then one would expect equiactive doses of physostigmine and neostigmine to have a similar effect on the action of nicotine. In this study equal doses of the two anticholinesterases were used. Bhattacharya & Feldberg (1958) have discussed the relative potencies of physostigmine and neostigmine in inhibiting true cholinesterase and pseudocholinesterase. As an inhibitor of mammalian pseudocholinesterase, the ratio of activity of physostigmine to neostigmine varied between 0.33 and 2.0 and as an inhibitor of true cholinesterase the range of activity varied between 0.5 and 4.0 according to the source of the enzyme and the investigator. The most frequently reported ratio was close to 1.0 and on this basis equal doses of the two drugs were assumed to be approximately equiactive. If neostigmine had a similar potentiating effect to physostigmine then this should have been apparent at the same dose. Physostigmine profoundly altered the effect of nicotine on bar-pressing behaviour but neostigmine did not. From this it can be concluded that the site of interaction of nicotine and physostigmine is central, since physostigmine is a tertiary compound which freely enters the brain whereas neostigmine is a quaternary compound which penetrates the blood-brain barrier less readily. The factors governing the entry of tertiary and quaternary compounds into the brain are discussed by Brodie & Hogben (1957).

The results of the present study are similar to the observations of Armitage, Milton & Morrison (1966) who showed that the ear-twitching caused by injection of nicotine into the lateral ventricle of anaesthetized cats was potentiated by intravenous physostigmine but not by intravenous neostigmine. Intraventricular neostigmine, however, did potentiate the ear-twitching. The most likely explanation of these effects is that nicotine acts by releasing acetylcholine within the brain. The potentiation by physostigmine of nicotine-induced behavioural depression suggests that this action of nicotine may also involve the release of acetylcholine.

It has been postulated that the arousal level of the brain is determined by the interaction of two mutually antagonistic systems (Hess, 1954). This hypothesis has been applied to the behaviour of rats by Carlton (1963) who postulated a cholinergic inhibitory mechanism which antagonizes the effects of a catecholamine-mediated arousal system. Physostigmine, by inhibiting cholinesterase, increases free acetylcholine levels (Goodman & Gilman, 1955) and also depresses behaviour (Pfeiffer & Jenney, 1957 ; Bovet, Robustelli & Bignami, 1965). The dose of physostigmine used in the present study was selected as having a threshold depressant effect on behaviour. It is possible that although this dose does not significantly depress performance at normal arousal levels it might be sufficient to counteract the stimulant effect of the lower doses of nicotine. The highest dose of nicotine normally causes a very brief behavioural depression and it is suggested that this is caused by release of acetylcholine which is rapidly destroyed by cholinesterase. In the presence of physostigmine the released acetylcholine would not be so rapidly destroyed and would therefore cause a prolonged depression. Experiments are at present in progress to test this hypothesis.

SUMMARY

1. Rats were trained to press a bar for water on a 2 min variable-interval schedule of reward. Nicotine (0.05, 0.1 and 0.2 mg/kg) injected subcutaneously increased the response rate while 0.4 mg/kg caused an initial reduction in response rate followed by a period of increased responding.

2. Pretreatment with the tertiary anticholinesterase physostigmine (0.05 mg/kg) potentiated the reduction in response rate caused by the highest dose of nicotine. The increase in bar-pressing caused by all doses of nicotine was reduced or abolished.

3. The quaternary anticholinesterase neostigmine did not alter the nicotine effect which implies that the site of interaction of nicotine and physostigmine was central.

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