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Effect of Scopolamine and Atropine on Habituation of the Flexor Withdrawal Reflex[1]

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PEARSON, J. A. *Effect of scopolamine and atropine on habituation of the flexor withdrawal reflex.* PHARMAC. BIOCHEM. BEHAV. 1(2) 155–157, 1973. –The effects of intraperitoneal injection of scopolamine and atropine on habituation of the flexor withdrawal reflex response in conscious rats were investigated. Scopolamine (200 and 500 μg/kg) caused a marked depression of reflex responses which habituated to a lesser degree than responses in control animals. Reasons are given for rejecting this finding as representing support for the hypothesis that habituation is dependent on cholinergic mechanisms. It was found that the establishment, but not the retention of habituation was impaired by administration of atropine (30 mg/kg).

Habituation Flexor-withdrawal reflex Scopolamine Atropine

IT HAS been suggested that habituation depends upon the integrity of muscarinic cholinergic mechanisms in the central nervous system [1]. This hypothesis has been supported by the results of experiments in which animals were allowed to adapt to an environment [2, 5, 7]. The degree to which adaptation had occurred was tested subsequent to the training or adaptation session. It was found that the degree of adaptation to the environment was reduced when cholinergic blocking agents (scopolamine or atropine) were administered prior to the training period. From the results of such experiments, the conclusion was made that scopolamine or atropine impaired the establishment of habituation. Whereas habituation may well be an example of adaptation to the environment, it is usually defined as decreased response to repeated stimulation. In the studies described above, the establishment of habituation was inferred but no direct measurements were made of diminution of response as a function of prolonged exposure to the stimulus. More recently, Warburton and Groves [9] have shown that habituation of startle responses to repeated auditory stimulation was not affected by prior administration of scopolamine.

Habituation of the flexor withdrawal reflex has been shown to possess characteristics identical to those of complex behavioural reactions [8]. It can thus be regarded as a suitable model on which to examine mechanisms responsible for habituation in general. Experiments reported here were carried out in order to determine the effect of cholinergic blocking drugs on habituation of the flexor reflex of the conscious rat.

METHOD

Male rats of the Long-Evans strain (weight range, 230–300 g) were anaesthetised with ether. EMG recording electrodes were inserted into the caudal head of the right biceps femoris muscle and stimulating electrodes were sutured into the skin of the right hind paw. After insertion of electrodes the rats were placed into Bollman-type restraining cages in which they remained until completion of the experiment which was carried out one day later.

Uniform electrical stimuli (15 V, 10 mA, 5 msec) were applied at 5 sec intervals by means of a Devices 2533 Isolated Stimulator. A Devices Digitimer was used to trigger the stimulator. Five hundred stimuli were given in each experiment. The EMG discharge evoked in response to each stimulus was monitored on a Tektronix 565 oscilloscope with a Tektronix 3A9 differential amplifier. Quantitative assessment of the discharge was achieved by means of an integration unit. Amplified EMG was integrated over a period of 250 msec after each stimulus. The integrated voltage was held for a period of 4 sec and was displayed on a digital voltmeter. Before and after each test period, values for basal EMG, also integrated over 250 msec, were obtained. The mean value for basal activity was subtracted from each value obtained in response to a stimulus to give net reflex response (expressed in volts). The mean response to successive groups of 10 stimuli was calculated.

Twenty minutes before the first stimulus an intraperitoneal injection of either saline, scopolamine (hyoscine hydrobromide) or atropine sulphate was given. The doses given were (a) scopolamine; 50, 200 or 500 μg/kg, and (b)

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atropine; 5, 10 or 30 mg/kg. These doses were chosen on the basis of results of earlier workers [2,9]. On each day four experiments were carried out: one in which saline was injected and three in which different doses of either scopolamine or atropine were given. This procedure was adopted so that any apparent change in response, as a result of administration of the drug, could not be attributed to daily variations in the stimulating and recording equipment.

RESULTS AND DISCUSSION

Mean values of flexor reflex responses (8 rats in each group) are illustrated in Figs. 1 and 2. Response levels are expressed both in absolute terms (above) and as percentages of the mean response to the initial 10 stimuli in each rat (below).

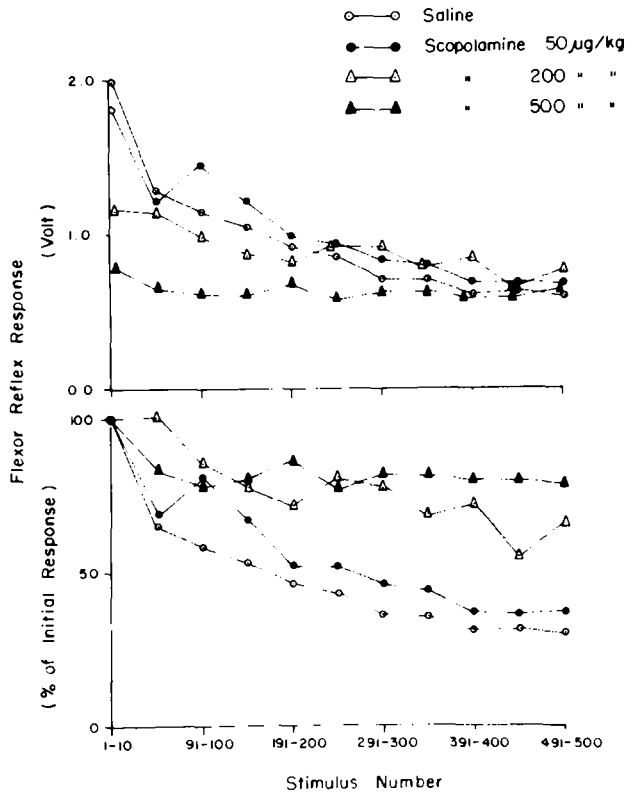

FIG. 1. Mean flexor reflex responses to stimuli presented at 5 sec intervals. Intraperitoneal injection of saline or scopolamine was given 20 min before the first stimulus.

Scopolamine (see Fig. 1, above) causes a depression of the flexor reflex. The responses to the initial stimuli in experiments in which 200 or 500 µg/kg of scopolamine were given, were significantly (t=3.06 and 3.13 respectively; $p<0.01$ in both cases) lower than the initial responses in control animals and animals which had received the lowest dose of scopolamine. Inspection of the data expressed as percentages of the initial response (see Fig. 1, below) reveals that there is an apparent impairment of habituation when the larger doses of scopolamine had been given. An alternative, and more likely, explanation is that in this preparation there is a response value which can be regarded

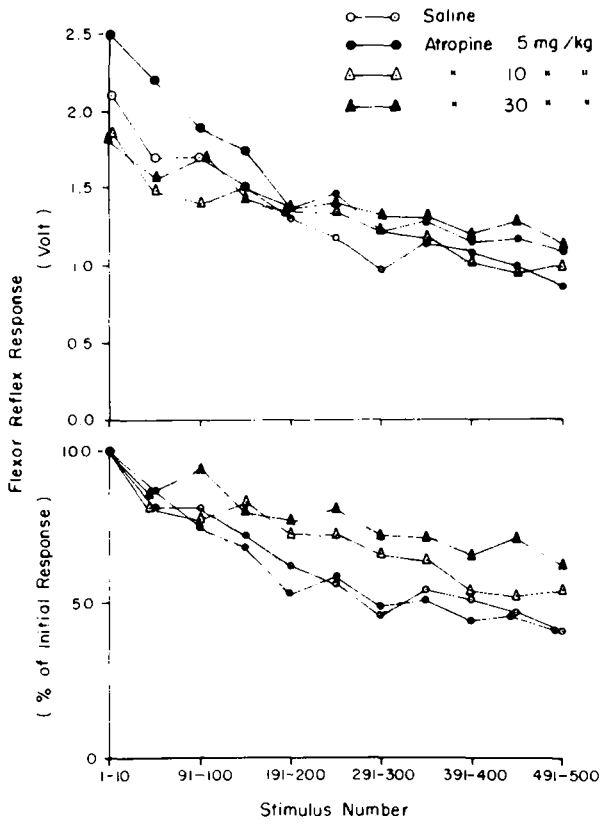

FIG. 2. Mean flexor reflex responses to stimuli presented at 5 sec intervals. Intraperitoneal injection of saline or atropine was given 20 min before the first stimulus.

as representing maximal habituation. When the response had been depressed almost to this value, as a consequence of scopolamine administration, very little further decrement could result from repeated application of the stimuli. These data can not therefore be used to support the hypothesis that habituation is dependent on cholinergic mechanisms [2].

Administration of atropine in doses which have potencies approximately equal to those of the doses of scopolamine used, did not result in significant ($p>0.10$) depression of reflex responses (see Fig. 2 above). When the data from these experiments were expressed as percentages of the initial response (Fig. 2 below) the mean responses to the 491st–500th stimuli, when doses of 5 or 10 mg/kg of atropine were given, were not significantly different ($p>0.05$) to the corresponding control value. In the experiments in which atropine was given in a dose of 30 mg/kg, the final response was significantly greater (t=2.74, $p<0.02$) than the control level. This impairment of response decrement took place even though the absolute level of responsiveness had not been depressed by the atropine. These data indicate that habituation of the flexor reflex, unlike the startle reflex [9] is at least partially dependent upon muscarinic cholinergic mechanisms.

Although Warburton and Groves [9] could not demonstrate any effect of scopolamine on the establishment of habituation, they did show that the retention of habituation was impaired in rats which had been treated with

scopolamine. Experiments were therefore carried out on two groups of rats (8 rats in each group) to determine if retention of habituation of the flexor reflex could be modified by the administration of a cholinergic blocking agent. Two series of 500 stimuli, which were separated by a pause of 2 hr, were given. In the control group, to which intraperitoneal saline was given 20 min before the first stimulus, the mean response amplitude during the second series was 78.0% ± S.E. 5.1 of the mean response to the first series. This suggests that the initial habituation had been partially retained and transferred to the second series of stimuli. In animals to which atropine (30 mg/kg) was given prior to the first series, the mean response during the second session was 88.8% ± S.E. 10.8 of the mean response during the first series. This value is not significantly different ($t = 0.9$, $p > 0.10$) from the corresponding response in the control experiments.

Atropine is not metabolised by the rat, but is excreted very slowly [3]. Approximately 25% of the injected atropine is excreted within the first 3 hr after intraperitoneal administration [4]. Moreover, there is a reasonably good correlation between the time course of decay of the central effects of atropine and the rate of excretion, i.e. 3 hr after a single injection of atropine the effectiveness of carbachol as a stimulant of drinking is reduced by only 20–30% [6]. In view of these findings it is reasonable to assume that in the present experiments the effect of atropine persisted throughout the interval between the series of stimuli and during the second series. It was not possible to demonstrate that retention of habituation to the first series of stimuli had been impaired by atropinization.

The data presented in this report indicate that the establishment but not the retention of habituation of the flexor withdrawal reflex is dependent upon atropine sensitive cholinergic mechanisms.

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