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Amnesia of a Passive Avoidance Task Due to the β_2-Adrenoceptor Antagonist ICI 118,551

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DAVIES, D. C. AND J. M. PAYNE. *Amnesia of a passive avoidance task due to the β_2-adrenoceptor antagonist ICI 118,551.* PHARMACOL BIOCHEM BEHAV 32(1) 187–190, 1989.—The selective β_2-adrenoceptor antagonist ICI 118,551 induces amnesia in the domestic chick when given systemically, 10 min after a one-trial PAL task. Young chicks will spontaneously peck at a small bright bead. If the bead has been coated with a distasteful substance, the chicks will learn in a single trial not to peck at a similar bead on subsequent presentation. Administration of ICI 118,551 prevented retention of this task. Vehicle-injected chicks which learnt the task, avoided a similar bead to the training bead in the retention test, but did not avoid a bead of a different colour. The effect of ICI 118,551 is unlikely to be a direct effect on performance since amnesic chicks pecked both beads freely and equally in the test.

Amnesia β_2-Adrenoceptor antagonist ICI 118,551 One-trial passive avoidance learning Chick

THE hypothesis that noradrenergic mechanisms facilitate learning and memory (4,16) by promoting the synaptic plasticity postulated to underlie these processes, has stimulated much research. Many studies have attempted to elucidate the role or noradrenaline in learning and memory, employing numerous paradigms and techniques in a variety of animals. However, these studies have not given universal support for a role of the noradrenergic system in learning and memory (17).

There is, however, considerable evidence for the involvement of noradrenergic mechanisms in learning and memory in the domestic chick. Noradrenaline is present in the chick forebrain at hatching and its concentration increases with both age and visual experience of an imprinting object (6). Furthermore, administration of the specific noradrenergic neurotoxin (15) N-(2-chlorethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP4) depletes chick forebrain noradrenaline and prevents the learning process of imprinting (7).

In another task, one-trial passive avoidance learning (PAL), the noradrenergic agonists noradrenaline, amphetamine (which among other things stimulates noradrenaline release), pargyline (a monoamine oxidase inhibitor) and the adrenergic receptor agonists methoxamine and isoprenaline, have been shown to reverse amnesia induced by the protein synthesis inhibitor cyclohexamide (11,13). More recently, a series of experiments was performed to evaluate the effects of various α- and β-adrenoceptor antagonists on one-trial (PAL) in the chick (24). Systemic injection of the α_1-adrenoceptor antagonist phenoxybenzamine, the α_2-antagonist piperoxane or the β_1-antagonist atenolol did not disrupt memory formation. However, the nonselective β-adrenoceptor antagonists sotalol, nadolol and timolol all impaired the acquisition of a one-trial PAL task. These three β-adrenoceptor antagonists have been shown to block both β_1- and β_2-adrenoceptors, but are not known to have any other side effects (3,9).

Since α- and β_1-adrenoceptor antagonists did not produce amnesia of the one-trial PAL task, and β-adrenoceptors in the chick brain are predominately or entirely of the β_2 subtype (18), these findings suggest that the nonselective β-adrenoceptor antagonists induced amnesia of the one-trial PAL task by their action at β_2-adrenoceptors. The present study was designed to examine this possibility by investigating the effect of the specific β_2-adrenoceptor antagonist ICI 118,551 (1,21) on one-trial PAL in the chick.

METHOD

Fertile 'Ross 1' eggs (Ross Poultry G.B. Ltd., Woodhall Spa, Lincolnshire, U.K.) were incubated and hatched as described previously (6). After hatching, chicks of either sex taken from the middle of the hatch (5) were transferred to individual compartments in a holding incubator maintained at a temperature of 32±2°C. The holding incubator was housed in a quiet room and illuminated by diffuse overhead light during normal working hours.

The chicks were trained in a one-trial taste aversion PAL task (2). Young chicks will spontaneously peck at a small, visually conspicuous bead. If the bead has been coated with

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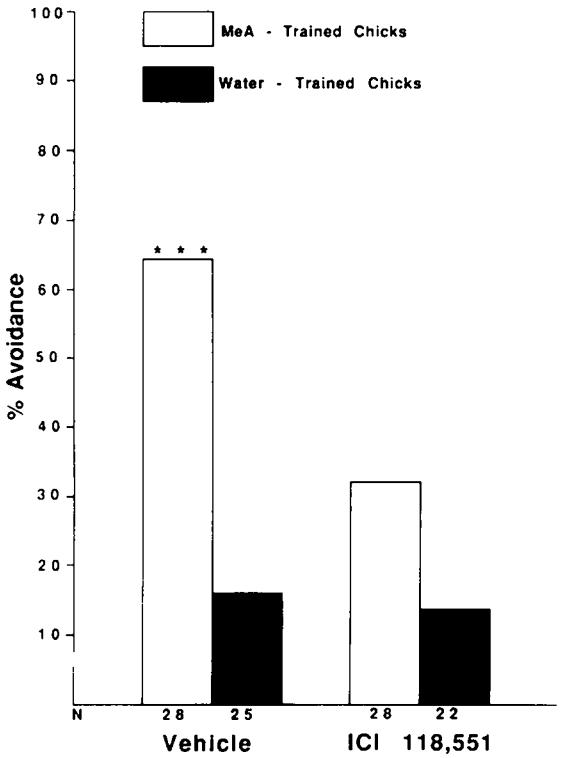

FIG. 1. Percentage of chicks avoiding a red bead in the retention test following administration of ICI 118,551 or vehicle 10 min after training. The retention test was performed 3 hr after training. Vehicle-injected chicks, trained on a MeA-coated bead, avoided the red test bead significantly more than did those chicks trained on a water-coated bead, ***$p<0.01$. There was no difference between ICI 118,551 injected chicks trained on either MeA or water in avoidance of the red test bead. N is given beneath each bar.

a distasteful substance such as methyl anthranilate (MeA), the chicks will not peck a similar but water-coated bead on subsequent presentation. In contrast, chicks which are trained on a water-coated bead will continue to peck such a bead. Thus, in a single trial chicks learn not to peck at an aversive stimulus.

At approximately one-day-old, each chick was transferred to a cylindrical arena 30.0 cm high and 14.5 cm in diameter and was allowed to adapt to its new environment for 5 min. A red bead (6.0 mm in diameter) dipped in either water or MeA was then presented to the chick through an aperture in the arena wall 15.5 cm above its base. The latency to the first peck was recorded and those chicks that did not peck the bead within 60 sec were excluded from further investigation. Chicks which pecked the MeA-coated bead showed one or more components of a disgust response, that is head shaking, beak wiping and eye closure. Following training, the chicks were replaced in the holding incubator.

Ten minutes after training, each chick received an intraperitoneal injection of either the selective β_2-adrenoceptor antagonist ICI 118,551 (16 mg/kg body weight in 0.1 ml distilled water) or a similar volume of vehicle. This time schedule for drug treatment was chosen since previous experiments (24) have shown nonselective β-adrenoceptor antagonists to induce amnesia on a one-trial PAL task when administered similarly. The relatively high dose of ICI 118,551 was used since inhibition binding assays showed it to have approximately half the binding affinity of the nonselective β-adrenoceptor antagonist timolol, which has been shown previously to induce amnesia of a one-trial (PAL) task (24).

TABLE 1

EFFECT OF ICI 118,551 ON BEAD COLOUR DISCRIMINATION

Training Substance	Treatment	N	% Avoidance at Test: Red Bead	% Avoidance at Test: Blue Bead
MeA	Vehicle	28	64.3*	28.6
MeA	ICI 118,551	28	32.1	17.9
Water	Vehicle	25	16.0	8.0
Water	ICI 118,551	22	13.6	18.2

Comparison of the percentage avoidance of a red bead (training colour) and a blue bead (previously unseen) in the retention test, given 3 hr after training. *$p<0.02$.

A retention test was given 3 hr after the end of training. The chicks were replaced in the arenas, allowed to settle for 5 min and presented with a red bead coated with water. Each chick was subsequently presented with a similar, but blue bead. The latency to peck the beads was recorded. Each bead was presented for a maximum of 30 sec and there was an interval of 5 min between presentation of the red and blue bead. Unpublished experiments in our laboratory have shown that the colour of the training bead (red or blue) does not effect the chicks' subsequent performance, neither does varying the order of presentation of the two beads at test. Therefore, for convenience, only one colour of bead was used for training and this was presented first at test. The retention test was performed 'blind,' the experimenters not knowing the chicks training substance or drug treatment. The chicks behaviour during both training and testing was viewed in a mirror suspended above the arenas and both procedures were performed under a fume hood to minimise olfactory effects of MeA. Following training, the gender of all chicks was determined using the 'feather-sexing' technique (14). Statistical comparison of the results was performed using the chi-squared test.

RESULTS

Figure 1 shows that significantly less ($p<0.05$) chicks injected with ICI 118,551 and trained on a MeA-coated bead (32.1%, n=28) avoided the red bead at test than did those chicks trained on MeA and injected with vehicle alone (64.3%, n=28). The avoidance of the red bead at test by these MeA-trained, vehicle-injected chicks was significantly greater ($p<0.01$) than that of vehicle-injected chicks trained on a water-coated bead (16.0%, n=25). There was no significant difference in avoidance of the red bead at test between chicks injected with ICI 118,551 and trained on either a MeA or water-coated (13.6%, n=22) bead. The avoidance of both groups of chicks injected with ICI 118,551 was similar to that of chicks trained on a water-coated bead and injected with vehicle. Therefore, MeA-trained, vehicle-injected chicks acquired the one-trial PAL task, but treatment with ICI 118,551 induced amnesia of the task.

All chicks showed low avoidance of the blue bead at test (Table 1). Moreover, there was no significant difference in avoidance of the blue bead between any of the groups of chicks tested. Chicks trained on a MeA-coated bead and

injected with vehicle avoided the red bead significantly more than the blue bead at test ($p<0.02$). Chicks trained on MeA and injected with ICI 118,551 showed no significant difference in avoidance of the two beads at test, neither did water-trained chicks injected with either ICI 118,551 or vehicle. Thus, those chicks trained on MeA and injected with vehicle that learned the task could discriminate between the bead on which they were trained and a similar sized bead of a different colour.

The latency to peck during training was not affected by the chicks' treatment or training substance. Neither was there any significant difference between the pecking of male and female chicks. Thus, all groups of chicks showed similar pecking activity.

DISCUSSION

The results of this experiment demonstrate that administration of the selective β_2-adrenoceptor antagonist ICI 118,551 impairs one-trial PAL in the chick. Previous studies (24) have implicated β-adrenoceptors in this form of learning in the chick and the current study provides evidence that β_2-receptors are the active subtype. This finding is consistent with the observation that the majority of the β-adrenoceptors in the chick brain are of the β_2 subtype (18).

Chicks trained on a MeA-coated bead and injected with vehicle learned not to peck the red bead at test. However, they subsequently pecked a similar, but blue bead. Thus, chicks can recognise the visual characteristics of the bead on which they were trained and associate an unpleasant gustatory experience with these characteristics. The amnesic effect of ICI 118,551 is unlikely to be due to visual impairment in this passive avoidance task, since amnesic chicks pecked both the red and blue beads freely at test. Neither is the drug likely to have a direct effect on performance since there was no significant difference in avoidance of either the red or blue bead at test by chicks trained on a water-coated bead and injected with ICI 118,551 or vehicle.

The blood-brain barrier is poorly developed in the young chick (23,26) and thus it is likely that the β_2-adrenoceptor antagonist ICI 118,551 passes freely into the brain, although its exact site of action is unknown. However, a variety of evidence (19,22) has suggested that a part of the chick forebrain, the medial part of the hyperstriatum ventrale, is involved in one-trial PAL. Furthermore, bilateral radiofrequency lesions of the intermediate part of the medial hyperstriatum ventrale (IMHV) prevent the acquisition of a one-trial PAL task (8).

Noradrenergic terminals have been visualised in IMHV by fluorescence histochemistry (unpublished observations). The presence of noradrenaline in this region has been confirmed by enzyme radiochemical assay and its concentration has been shown to increase with both age and visual experience over the first 50 hr posthatch. Furthermore, β-adrenoceptor binding capacity in IMHV increases over the first 48 hr posthatch (20), and thus the noradrenergic system in IMHV is labile over the period during which the one-trial PAL task is normally performed.

In the model of memory formation in the chick formulated by Gibbs and Ng (12), long-term memory is dependent on protein synthesis and thus its formation can be prevented by protein synthesis inhibitors. Morphological synaptic changes have been observed in the medial hyperstriatum ventrale following one-trial PAL (25). Similar changes have been observed in IMHV and were prevented by administration of the amnestic protein synthesis inhibitor anisomycin (10). Thus, it would appear likely that protein synthesis is at least in part associated with the synaptic changes that occur in IMHV following one-trial PAL. Since the selective β_2-adrenoceptor antagonist ICI 118,551 induces amnesia of the one-trial PAL task and the noradrenergic system is labile shortly after hatching, it is possible that the β_2-adrenoceptor in some way mediates the synaptic changes which may underlie long-term memory formation.

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