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Amnesia Due to β-Antagonists in a Passive Avoidance Task in the Chick

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STEPHENSON, R. M. AND R. J. ANDREW. *Amnesia due to β-antagonists in a passive avoidance task in the chick.* PHARMAC. BIOCHEM. BEHAV. 15(4) 597–604, 1981.—The β-adrenergic antagonists sotalol, nadolol and timolol (which act at both β_1-and β_2-receptors) induce amnesia in the domestic chick when given systemically after a one trial passive avoidance task. State dependent learning and effects on performance at test (e. g. interference with recall) almost certainly do not cause the observed amnesia. All three effective β-antagonists induce amnesia only when injected within a limited period after training, suggesting effects on memory formation. Sotalol differs markedly from nadolol and timolol in: (a) showing a sharp and markedly earlier loss of effectiveness as administration is moved to progressively later times after training (25–30 min rather than 40–50 min), (b) producing when given after training a delayed and gradual loss of retention rather than an immediate and rapid loss. These differences seem not to reflect a lesser effectiveness of sotalol, nor a greater delay in the onset of its action, but instead qualitative differences in effects on memory formation.

Amnesia Sotalol Nadolol Timolol Single trial passive avoidance learning β-antagonists

A NUMBER of lines of evidence suggest that both central and peripheral noradrenergic/adrenergic systems may be important in learning and memory formation. Disruption of the noradrenergic supply to the cat visual cortex prevents normal experiential changes in visual units, whilst the local infusion of noradrenaline restores such plasticity in the area of infusion [30]. The dorsal noradrenergic bundle has been held to be crucial in reward and subsequent learning [8, 36, 42], although its role remains a matter for considerable debate [7, 23, 37].

In mammals, Kety's hypothesis [20] that the release of noradrenaline promotes long term memory formation has in general been supported by experiments in which drugs are given after training. Thus the depression of central noradrenaline levels is sometimes [12, 24, 32, 34] associated with poor retention. However, this is not always so: small doses of alpha-methyl-paratyrosine (30 mg/kg), adequate to depress central tyrosine hydroxylase activity, interact in a complex fashion with footshock intensity in influencing retention of a passive avoidance task in mice [17]. Retention of avoidance training is promoted at low levels of footshock but there is disruption at higher levels. Another complication is that it is not always certain that it is changes in noradrenergic function which underlie retention deficits. For example, it is unclear whether the amnesic effects of powerful copper chelating agents such as diethyldithiocarbamate results from inhibition of dopamine hydroxylase activity or one of a number of non-specific side effects [18].

However, recent work utilising more specific disturbance of neurotransmission at identified receptor subtypes supports the idea that noradrenergic mechanisms play an important role in memory consolidation. Noradrenergic agonists may oppose amnesia associated with depression of noradrenaline levels [10]. In addition, both α and β-agonists have been shown to oppose amenia caused by agents which inhibit protein synthesis [31]; this has also been reported for chicks using a similar task to that used here [14]. Post-trial injection of the noradrenergic antagonists propranolol and alprenolol have produced amnesia in a passive avoidance task in rats [6, 13]. Unfortunately, the evidence is complicated by the fact that propranolol has also been found to facilitate learning [25]. Recently noradrenergic antagonists have become available which not only have a high degree of specificity but also lack of complicating side effects, such as membrane stabilisation and sympathomimetic effects [5, 11, 21]. Here we investigate the effect of such specific antagonists upon memory in the chick.

EXPERIMENT 1: RELATIVE EFFECTIVENESS OF VARIOUS α- AND β-ANTAGONISTS

In this experiment a number of specific α- and β-antagonists were given either before or after training at a standard and relatively high dose [4 mg/kg]. Dosage response curves for those agents which proved to be effective are given later (Experiment 3): since effectiveness was found to vary markedly with time of injection after training, and to differ between agents in the way in which this occurred, it was found necessary first to establish time courses of loss of effectiveness (Experiment 2).

METHOD

Male Warren Sex-link chicks (weight 50 gm) were housed in pairs (to avoid stress due to isolation) on arrival from the hatchery. Cages were 18 cm by 25 cm by 20 cm, painted a matt grey inside and illuminated from above by a 25 W

TABLE 1

EFFECT OF α- AND β-ANTAGONISTS ON RETENTION*

	Time of Administration			
	5 min before		10 min after	
	% Not Pecking		% Not Pecking	
Treatment†	Aversive	Neutral	Aversive	Neutral
Vehicle	79	15	70	16
Sotalol: $\beta_1 + \beta_2$	18¶	5	23‡	6
Timolol: $\beta_1 + \beta_2$	37‡	16	29	16
Atenolol: β_1	80	0	74	5
Piperoxane: α_2	53	5	75	30
Vehicle	79	21	75	18
Nadolol: $\beta_1 + \beta_2$	29§	5	16§	16
Phenoxybenzamine: α_1	74	21	78	11

*Retention is shown as the percentage of individuals which did not peck (% Not Pecking) the bead of the type used in training (red: Aversive) at a retention test 180 min after training. Retention is also shown for a bead (blue) of a type not previously seen (Neutral). Each treatment group was of 16–20 birds.

†Sotalol, timolol, atenolol and piperoxane (4 mg/kg, in 50 μl 154 mM NaCl) and nadolol and phenoxybenzamine (4 mg/kg in 10 μl dimethylacetamide) were given at 5 min before, or 10 min after training. Statistical comparison (χ_1^2) was with the control group (Vehicle) receiving the same volume of the same vehicle at the same time of administration.

‡$p<0.02$, §$p<0.01$, ¶$p<0.001$.

tungsten lamp. One bird in each of the pairs was marked on the head with black ink so that individuals could be distinguished at test.

The passive avoidance task which was used, has been employed in earlier chick studies [6, 22, 38]. Our procedure followed closely that described by Gibbs and Ng [15]. It began with four presentations of a bead, wet with water and mounted at the end of a stiff wire (pretraining). The first two were of a small white bead (plastic, 3 mm in diameter). Its small size reduced avoidance; this preliminary experience made it less likely that the bead presented at training would be avoided. Presentation of a red and then a blue bead followed (colored glass, 6 mm in diameter). The pretraining presentations each lasted about 10 sec, and were separated by 3 min. The pair of chicks often pecked simultaneously at the bead; if they did not, each was presented in turn with the bead, held about 3 cm in front of its bill tip. Training followed after 120 min: a red bead identical in appearance with that used in pretraining but coated with the distasteful substance methyl anthranilate was presented. Presentation was continued until both birds had ceased to peck and had shown one or more responses indicating they had tasted the methyl anthranilate (head shake, eye closure, with upward tilt of the bill). This typically occurred within 5 sec. A retention test followed after 180 min: a red, and then a blue bead of the type used in pretraining were presented, each for 10 sec with an interval of 5 min. Pecking was recorded by a hand-held keyboard, with a key for each chick.

Drugs were injected subcutaneously with a Hamilton repeating syringe into a fold of abdominal skin. Three β-antagonists, affecting both β_1- and β_2-receptors (sotalol: Glaxo; nadolol: Squibb; timolol: Merck, Sharp and Dohme), a β_1-antagonist (atenolol: ICI), an α_1-antagonist (phenoxybenzamine: Smith, Kline and French) and an α_2-antagonist (piperoxane: May and Baker) were used. They were dissolved in 50 μl 154 mM NaCl (sotalol, 4 mg/kg; timolol, 4 mg/kg; atenolol, 4 mg/kg; piperoxane, 4 mg/kg) or 10 μl dimethylacetamide (nadolol, 4 mg/kg; phenoxybenzamine, 4 mg/kg). The same vehicles were also used in all subsequent experiments.

All treatment groups were initially 20 birds; birds failing to peck at training were excluded, reducing group sizes to a minimum of 16. Each drug was given to one group 5 min before training and to another 10 min after training. The two control groups were injected with one or other of the vehicles alone. Statistical comparisons were made with the control group receiving the appropriate vehicle, using the number of chicks pecking the red bead at test in the χ^2 test for independent samples; expected cell sizes were appropriate for such tests [32].

RESULTS

All three β-antagonists (sotalol, nadolol and timolol, 4 mg/kg) which are effective in mammals at both β_1 and β_2-receptor sites [5, 11, 21] markedly and significantly reduced retention, whether given 5 min before or 10 min after training (Table 1). At the same dose a β_1-antagonist (atenolol), an α_1-antagonist (phenoxybenzamine) and an α_2-antagonist (piperoxane) [40] had no effects on retention, whether given before or after training. The neutral blue bead which had been seen in pretraining but had not been used in training was pecked freely by all groups (Table 1). This suggests that none of the drugs effective in opposing retention at 180 min after training did so by a general depressive effect on performance.

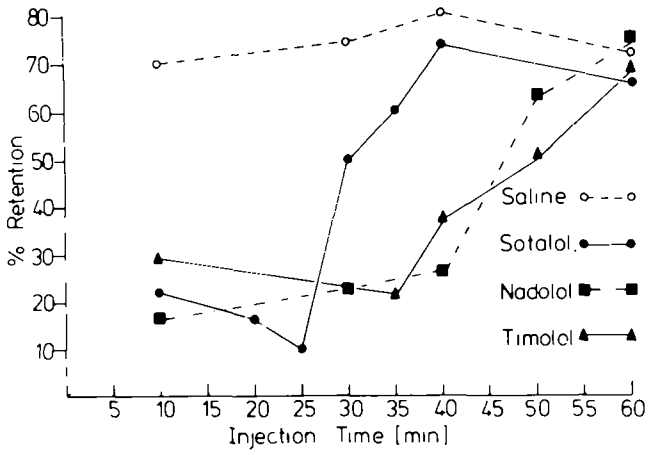

FIG. 1. Percentage of birds failing at test to peck the bead used in training (% Retention) when amnesic agents (sotalol, nadolol and timolol, all at 4 mg/kg) were given at times (Injection Time) between 10 and 60 min after training (Experiment 2). Retention test was 180 min after training. Each point is based upon a group of 16–20 birds.

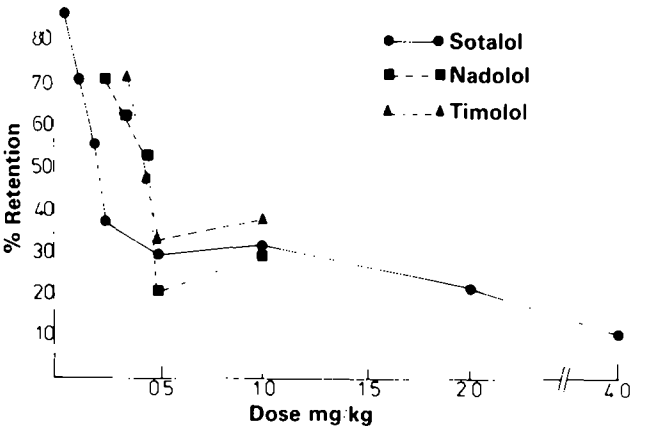

FIG. 2. Percentage of birds failing at test to peck the bead used in training (% Retention) when sotalol (0.1–4.0 mg/kg), nadolol (0.25–1.0 mg/kg or timolol (0.35–1.0 mg/kg) was injected 25 min after training. (Experiment 3). Retention test was 180 min after training. Each point represents a group of 16–20 birds.

EXPERIMENT 2: β-ANTAGONISTS INJECTED AT DIFFERENT TIMES AFTER TRAINING

In Experiment 1, β-antagonists given after training could have affected retention tests either by direct effects on performance at test or by effects on memory formation; if the latter were the case, it would be expected that the antagonists would be effective in disrupting subsequent retention for only a limited period after training.

METHOD

The subjects and procedure were similar to those of Experiment 1, except that different groups were injected at times extended over a period from 10–60 min after training, in steps of 5 or 10 min, with one of the three effective β-antagonists (sotalol, nadolol, and timolol: all at 4 mg/kg; vehicles and volumes as in Experiment 1). In addition to χ^2 tests, a method taken from quality control analysis [39] was used to provide an objective measure when comparing times after training at which different antagonists ceased to be effective. Individual pecking rates on the red bead at test were analysed. Points in time soon after training, when injection of the antagonists produced standard large retention deficits were taken as representing a steady state. Each successive point over progressively longer intervals after training was then compared with this standard, until a point differing from it by more than two standard deviations was encountered (warning point). A formal comparison (t-test) was then made between the standard state and the next point (action point). The standard state was based upon the first three points for sotalol and nadolol (n=53 and 56) and the first two for timolol (n=37).

RESULTS

All three effective β-antagonists affected retention tests only when given within a limited period after training. Unexpectedly, the duration of this period differed between antagonists: there appeared to be a relatively sharp loss of sensitivity to sotalol between 25 min after training (when it was maximally effective) and 35–40 min after training with most of the change occurring between 25–30 min; sensitivity to both nadolol, and timolol began to fall away later at around 35–40 min after training and was not fully lost until 60 min (Fig. 1). When injection was at 10 min after training the groups receiving sotalol, nadolol and timolol all differed significantly from the matched control group ($p<0.02$, $p<0.01$, $p<0.02$, respectively, χ_1^2). When injection was at 40 min after training the nadolol and timolol groups still differed significantly from the control group ($p<0.001$ and $p<0.01$, respectively, χ_1^2). The warning point for sotalol was 30 min, as against 50 min for nadolol and timolol. All three comparisons between the standard state, resulting from injections close to training, and the action point were significant (sotalol: 35 min, $p<0.01$; nadolol: 60 min, $p<0.001$; t-test).

These data suggest that, at the doses used, loss of sensitivity to sotalol follows a time course significantly different from that shown by the other two agents.

EXPERIMENT 3: DOSAGE CURVES FOR SOTALOL, NADOLOL AND TIMOLOL

The most obvious explanation for the above results (ie. the earlier loss of effectiveness of sotalol) is that it is a weaker β-antagonist than nadolol and timolol: this is probably the case for mammalian peripheral β-receptors tested *in vitro* [5,11]. If this were also the case for the effect under discussion, then even within its period of effective action sotalol should cease to act at higher doses than the other two agents. A range of doses of all three antagonists were therefore compared. These were given at the latest time (25 min) after training at which even the high dose of sotalol so far used was known to be effective. Any shift to the left of the point of inflection of the sotalol curve with falling dosage should be revealed in this experiment by a loss of effectiveness at a higher dosage of sotalol than of the other two agents.

TABLE 2

β-ANTAGONISTS GIVEN AT TRAINING AND AT TEST

Treatment*	% Not Pecking	
	Aversive	Neutral
Sotalol-sotalol	28†	5
Vehicle-sotalol	79	26
Nadolol-nadolol	22†	17
Vehicle-nadolol	75	6

*Treatment before training is shown first followed by treatment before test. Statistical comparison (χ_1^2) was between groups receiving the same antagonist at test.

†$p < 0.001$.

TABLE 3

RETENTION 24 HRS AFTER TRAINING

Treatment*	% Not Pecking	
	Aversive	Neutral
Nadolol	22†	5
Timolol	26†	6
Sotalol	21†	21
Saline	80	10

*Injection was 25 min after training. Statistical comparison (χ_1^2) was with the saline controls.

†$p < 0.001$.

METHOD

The subjects and procedures were similar to those of Experiment 1, except that a range of doses were used (0.1–4.0 mg/kg, sotalol; 0.25–1 mg/kg, nadolol; 0.35–1 mg/kg, timolol). Vehicles, volumes and route of administration were unchanged; injection was at 25 min after training.

RESULTS

At 25 min after training sotalol is more, rather than less effective than the other two β-antagonists. Both timolol and nadolol show a sharp loss of effectiveness at doses just below 0.5 mg/kg, this being almost or quite complete at 0.35 mg/kg, whereas loss of effectiveness of sotalol begins at about 0.25 mg/kg and is not complete until about 0.125 mg/kg (Fig.2). At 0.25 mg/kg the sotalol injected group differed significantly from the corresponding nadolol group ($p<0.01$, χ_1^2): it also differed from a matched saline injected control group ($p<0.01$, χ_1^2).

EXPERIMENTS 4 and 5: POSSIBLE EFFECTS AT TEST OF β-ANTAGONISTS

Although Experiment 2 strongly suggests that the antagonists are acting during memory formation, it remains possible that they also directly affect behaviour at test. It is also possible that state dependency [2,27] might be involved: that is, learning (here, formation of the trace after learning) in a special physiological state induced by a drug might result in memory accessible only when the same physiological state is again produced. Both possibilities were examined in Experiment 4. In Experiment 5, groups injected at a time when the antagonists are fully effective were tested 24 hrs later to examine further the possibility of drug effects at test and to see how permanent was the induced amnesia.

METHOD

The subjects and procedures were similar to Experiment 1, except that each group was injected at 5 min before training and at 5 min before test (Experiment 4) ot at 25 min after training (Experiment 5); Experiment 5 also differed in that retention was tested after 24 hrs. Vehicles, volumes and route of administration were the same in both cases as in Experiment 1. In Experiment 4, four combinations of injections were used at training and test: sotalol-sotalol, nadolol-nadolol, vehicle-sotalol, vehicle-nadolol. The dosages were 4 mg/kg (sotalol), and 1 mg/kg (nadolol), since it was thought at the time that nadolol was the more effective agent: the relative effectiveness of these two agents were given at 5 min before training in fact remains to be established. In Experiment 5 nadolol, timolol and sotalol were used, all at 1 mg/kg.

RESULTS

In Experiment 4 the two groups receiving a β-antagonist at both training and test showed a degree of disruption of retention which would have been expected if the antagonist had been given only before training [Table 2]. The group receiving a β-antagonist only at test showed no disruption of retention and differed significantly from the corresponding drug-drug groups. All groups pecked the neutral bead freely.

In Experiment 5 nadolol, timolol and sotalol given 25 min after training marked loss of retention at a test 24 hrs after training (Table 3). All differed significantly from the controls. It is known (Stephenson, unpublished observations) that dimethylacetamide alone (vehicle:nadolol) does not affect retention at 24 hrs. All groups pecked the neutral blue bead freely.

EXPERIMENTS 6, 7 AND 8: TIME COURSES OF LOSS OF RETENTION AFTER β-ANTAGONISTS

The time course of loss of retention after the administration of an agent effective in disrupting retention differs in marked and interesting ways between different types of agents in the chick [15]. In view of the differences already found between sotalol, on the one hand, and nadolol and timolol on the other, such time courses were examined for these three agents.

METHOD

The subjects and procedures were the same as in Experiment 1, except for injection times (5 min before training: Experiment 6; 15 min after training: Experiment 7; 10, 15; and 25 min after training: Experiment 8) and timing of the retention test, which was given at 5, 10, 15 and 180 min after training in Experiment 6, at 20, 30, 40, 45, 55, 60 and 180 min in Experiment 7 and at 40, 50 and 60 min in Experiment 8. Here, as in all other experiments, each point represents a different group of 16–20 birds. Sotalol, nadolol and timolol were compared in Experiments 6 and 7, whilst sotalol alone

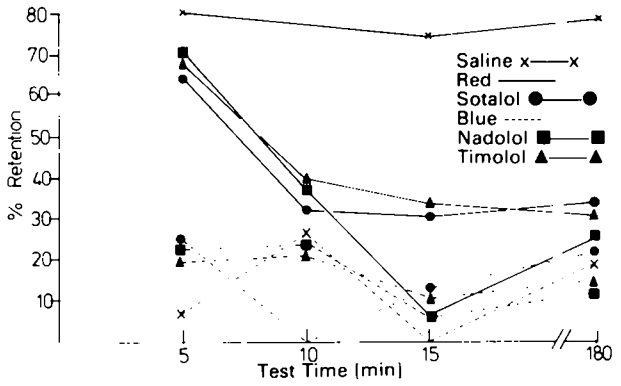

FIG. 3. Percentage of birds failing at test to peck the bead used in training (% Retention) with tests at intervals following training (Test Time) of 5, 10, 15 or 180 min (Experiment 6). Sotalol, nadolol or timolol (1 mg/kg) or saline was injected 5 min before training. Each point is based on a group of 16–20 birds. Response to the aversive bead (Red) and to the neutral bead (Blue) is shown by continuous and broken lines, respectively.

was used in Experiment 8; all were 1 mg/kg. Comparisons were made between antagonist injected groups and control groups receiving 154 mM NaCl. Dimethylacetamide groups were not included but earlier work (Stephenson, unpublished observations) indicated that this agent had no effect upon retention tested over a similar range of time after training.

RESULTS

After injection at 5 min before training (Experiment 6) all three β-antagonists produced a similar rapid loss of retention (Fig.3) At 5 min after training retention was almost or quite complete, whilst at 10 min there was little retention. Retention levels did not fall further between 10 and 180 min, at which time saline injected controls differed significantly from groups receiving each of the three antagonists in all three cases ($p<0.001$, χ_1^2). At all times of testing the neutral bead was pecked freely (Fig. 3).

Injection at 15 min after training (Experiment 7), however, once again revealed a marked difference between sotalol and the other two β-antagonists. Loss of retention was considerably delayed in groups receiving sotalol: the retention curve did not begin to diverge obviously from saline injected controls until 45 min after training, and did not show full loss until 60 min after training (Fig. 4). The groups receiving nadolol and timolol already showed some sign of loss at 20 min after training. Loss was complete at 30 min, when for the first time nadolol and timolol groups differed significantly from the matched corresponding control group ($p<0.001$ and $p<0.01$, χ_1^2, respectively). At 180 min after training, as expected from earlier experiments, sotalol, nadolol and timolol groups all differed significantly from controls ($p<0.03$, $p<0.01$, and $p<0.001$, χ_1^2, respectively). At 20, 30, 40 and 180 min after training the neutral bead was pecked equally readily (Table 4), irrespective of response to the aversive bead.

Thus the time course of loss of retention in relation to the time of injection did not differ markedly in the case of nadolol and timolol between injection at 5 min before training (Experiment 6) and at 15 min after training (Experiment 7); in both cases loss was largely or quite complete 10–15 min after injection. This was not true of sotalol.

No further shift in the time course of loss of retention could be produced by further variation in the time of injection of sotalol (Experiment 8). Injection at 10, 15 and 25 min after training (the latter being the latest time at which sotalol was expected to act at all) produced curves similar to each other (Fig. 5), and to that independently obtained in Experiment 6 (Fig. 4). In all three series, at all times of testing the neutral bead was pecked freely (Fig.5). Indeed, the group which had been injected 25 min after training, showed at 60 min after training, retention intermediate between that shown by the 10 and 15 min groups. It thus seems unlikely that the delay in loss of retention following sotalol reflects a substantially slower onset of action at a cellular level than occurs with nadolol or timolol. If this were so, then the curve for the 25 min injection would be expected to be shifted to the right in comparison with the 10 min injection curve and it is not.

TABLE 4

RESPONSE TO NEUTRAL BEAD AFTER β-ANTAGONISTS

	% Not Pecking Neutral			
Min after training Treatment*	20	30	40	180
154 mM NaCl	18	0	11	16
Sotalol	21	16	6	15
Nadolol	21	21	13	5
Timolol	24	13	6	5

*Injection was 15 min after training.

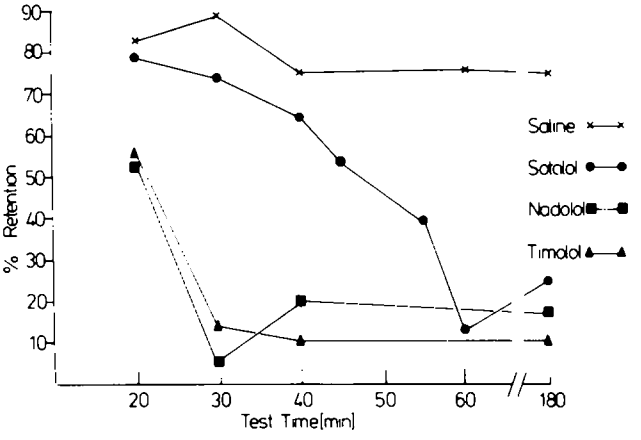

FIG. 4. Percentage of birds failing at test to peck the bead used in training (% Retention) with tests at 20, 30, 40, 45, 55, 60 or 180 min after training (Test Time: Experiment 7). Sotalol, nadolol or timolol (1 mg/kg) or saline was injected 15 min after training. Each point is based on a group of 16–20 birds.

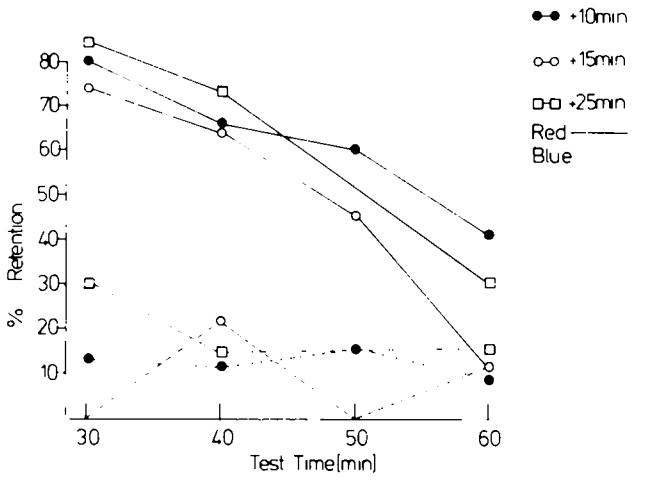

FIG. 5. Percentage of birds failing at test to peck the bead used at training (% Retention) with tests at 30, 40, 50 and 60 min after training (Test Time: Experiment 8). Sotalol (1 mg/kg) was given at 10, 15 and 25 min after training. Each point represents a group of 16–20 birds. Response to the aversive bead (Red) and to neutral bead (Blue) is shown by continuous and broken lines, respectively

GENERAL DISCUSSION

The data presented here strongly suggest that a number of β-antagonists disrupt memory formation in the chick. Thus, these antagonists are only effective when given after training in clearly defined and relatively short periods following training (Experiments 1 and 2). Outside these periods they are relatively ineffective, and continue to be ineffective when given just before the retention test (Experiment 4). Direct effects upon performance at test thus are unlikely to be responsible: not only is pecking at the bead of the type used in training normal in such groups, but the neutral bead is pecked freely.This is true even when β-antagonists are given just before testing (Experiment 4). Further, birds injected 25 min after training show retention deficits 24 hrs later (Experiment 5) at a time when testing could not reasonably be assumed to be affected by a significant concentration of the β-antagonist. This result also shows that there is no rapid recovery from amnesia due to β-antagonists.

State dependent learning can probably also be excluded in that birds showed the expected retention deficit when treated with the β-antagonists before both training and testing (Experiment 4).

The three β-antagonists which are effective, all show broad opposition to β_1- and β_2-receptors, and are not known to have other side effects [5, 11, 21]. At similar doses α_1-, α_2-, and β_1-antagonists [5] were ineffective (Experiment 1), further suggesting relatively specific action, perhaps at β_2-receptors. This is consistent (given a central site of action) with the finding that β-receptors in the chick brain are predominantly or entirely β_2 in type [26].

Disturbance of noradrenergic (or adrenergic) function by β-antagonists might affect memory formation by a central or a peripheral route [29]. Although it is not known how freely β-antagonists pass the blood brain barrier in the young chick, its ineffectiveness as a barrier to other classes of agent [34] suggests that β-antagonists also might enter. Sotalol and timolol are effective when given intracranially (Stephenson, unpublished observations); however, despite the small volume used (1 μl), it could be argued that this is due to escape from the brain. The strongest support for central action come from another β_1 + β_2-antagonist, propranolol, which is known to be ineffective in producing amnesia in the chick when given systemically [14]. This seemed a serious discrepancy with our findings, since the retention task used in these experiments was very similar to ours. However, although propranolol also proved relatively ineffective in our hands, when given systemically, it is effective when given intracranially (Stephenson, unpublished observations). The high binding affinity for serum albumin of propanol [41] may explain its ineffectiveness when given systemically. In any event it seems very unlikely that leakage of propranolol into the systemic circulation after intracranial injection could be effective peripherally.

The main issue which we wish to discuss is the evidence that sotalol appears to affect memory formation in a way qualitatively different from nadolol and timolol. Sotalol differs from the other two agents, firstly, in ceasing to be effective when given more than 25 min after training. The transition is sharply defined and clearly earlier than the time (about 40 min after training) at which the other two agents begin to be less effective. It is not obviously sensitive to dosage: sotalol is effective at 25 min at doses clearly below threshold for nadolol and timolol (Experiment 3), whilst conversely it is ineffective at 40 min at a dose at which the other two agents are fully effective (Experiment 2). The second obvious difference is that retention is lost only after delay, and then gradually, when sotalol is given after training, whereas it is lost rapidly and almost immediately after nadolol and timolol (Experiment 7). This delay is not due to a slow onset of action, since when given before training sotalol acts as quickly as nadolol and timolol (Experiment 6). Further, when sotalol is given after training the time course of loss of retention is not shifted by changes in the time of injection (Experiment 8); its timing appears instead to be determined by the time of training.

Such clear differences presumably require some distinction in action at the cellular level. Such action might differ centrally because: (a) There are actually two subtypes of β_2-receptor in the chick brain, differentially responsive to two types of β-antagonists; the classification of β-receptors is based on mammaliam receptors and may not be complete even for these [2,36]. Chick erythrocyte β-receptors have recently been shown [9] to be divisible on their response to antagonists into subtypes which do not correspond to the β_1 and β_2 division in mammals. (b) The β-antagonists compete differently with different transmitters at the same type of β-receptor; adrenaline, as well as noradrenaline, appears to be important in the avian brain [19,26]. (c) One (or both) of the two types of effective β-antagonists have as yet undescribed side effects. The possibility of disturbance of memory formation by peripheral effects raises the further complication that one type of β-antagonist might act predominately centrally and one peripherally.

Fortunately it is not necessary to decide which cellular route of action is involved before considering further the evidence which the two types of β-antagonist provide about processes responsible for memory formation in the chick. In order to do this it is necessary also to consider the model of memory formation in the chick set out by Gibbs and Ng [15], using the same task as in the present study. They argue for

three sequentially dependent phases of memory formation, each of which can be blocked by a particular class of agent (agents affecting hyperpolarisation of neurons, the Na/K pump and protein synthesis). It would be premature to try to accomodate the data from β-antagonists with this model in detail, but we will see that it may require some extension and change.

Sotalol will be considered first. The relatively sharp loss of sensitivity with injections at intervals greater than 25 min after training is of particular importance since there is independent evidence [1] of a rather sharp transition in memory formation at a time 25–30 min after learning. When two contradictory experiences are given under appropriate circumstances, using a stimulus of the same type at each, there is at subsequent retention tests evidence of marked interference between the two experiences. The interval between the experiences is crucial in determining the outcome: as it lengthens, the first experience suddenly become immune to interference by the second at an interval of about 30 min ([1], and Clifton *et al.*, in preparation). The coincidence between these two estimates of the timing of a hypothetical sharp transition agrees with evidence (Stephenson, unpublished observations) that sotalol begins to act very soon after systemic injection.

The model of memory formation in the chick set out by Gibbs and Ng [15] assumes that protein synthesis inhibitors prevent the establishment of long term memory; the persistence of memory up to 30 min after learning followed by gradual decay, which occurs in the presence of such inhibitors [15] is then due to persistence of a trace in a phase which precedes long term memory. The time course of loss of retention when sotalol was given somewhat after training in the present study corresponds quite well with that produced by protein synthesis inhibitors. It could therefore be arged that sotalol also prevents the establishment of long term memory.

Protein synthesis inhibitors show a gradual loss of effectiveness when injected at progressively longer intervals after learning which is different from that shown by sotalol: it approaches completeness at 20 to 30 min. However, it is possible that this difference reflects differences at the cellular level of action, and that both types of agent block the establishment of long term memory, although by very different routes.

On any hypothesis it is necessary to assume that sotalol (unlike other agents used in the chick) has another and quite different effect when given before training, since loss of retention is then rapid. It is as yet impossible to say which out of the many processes which must occur during or immediately after learning, sotalol may affect.

Nadolol and timolol are remarkably effective by comparison with agents used previously in the chick. Thus they still disrupt retention when given as late as 40 min after training: if, as we have just argued, there is an important step in the establishment of long term memory around 30 min after learning, then disturbance is still possible for some time subsequent to it. The rapid loss of retention following injection of the agents as late as 15 min after learning is also unexpected. This finding in particular suggests effects on processes in memory formation markedly different from those affected by sotalol or the agents used by Gibbs and Ng; evidence bearing on the nature of these processes will be presented elsewhere.

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