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The Effects of 5-HT Receptor Blockade on Memory Formation in the Chick: Possible Interactions Between β-Adrenergic, and Serotonergic Systems

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STEPHENSON, R. M. AND R. J. ANDREW. *The effects of 5-HT receptor blockade on memory formation in the chick: Possible interactions between β-adrenergic and serotonergic systems.* PHARMACOL BIOCHEM BEHAV **48**(4) 971-975, 1994.—An antagonist, 21-009, with high affinity for 5-HT_{1B} receptors and some for β-adrenergic receptors, induces amnesia in the domestic chick when given centrally before training, using a one-trial passive avoidance task. Nonspecific behavioral effects, or effects upon performance at test, almost certainly do not cause the observed amnesia. The 5-HT_2 antagonist ketanserin and the 5-HT_3 antagonist ICS 205-930, did not have any effects when given before training at the same dose as that at which 21-009 was effective. The unusual pattern of action of 21-009 (sensitivity only at, or around the time of learning, combined with very delayed memory loss) resembles that of certain β-antagonists, but not others. The results are discussed in terms of both direct action on 5-HT systems involved in memory formation and possible interactions between 5-HT and β-adrenergic systems.

Chick Amnesia 5-HT β-Adrenoceptors Sotalol 5-HT_{1B} receptors Delayed memory loss

PREVIOUS work in this laboratory (24) and elsewhere (9) has established that β_2 antagonists are amnestic when administered to chicks, either systemically or directly to the brain, before or after learning a one-trial passive avoidance task. This has been argued to imply that central noradrenergic systems play a crucial role in chick memory formation (9,24,25), which would be consistent with evidence from studies of imprinting in the chick (8). In the rat, changes occur in central noradrenaline turnover immediately after the acquisition of a one-trial passive avoidance task (12), and both central and peripheral β-adrenergic systems appear to be involved in memory formation; interactions with other systems, particularly cholinergic (5,15) are clearly important. It was not possible to explain the full range of effects on memory formation, of the β-antagonists used in our earlier experiments, by a single mechanism of action within the central nervous system. Instead, such agents were divisible into two groups by their pattern of amnestic action in the chick. One group (Nadolol and Timolol) appeared to affect a process that is necessary for retrieval, for at least 40 min after training, strongly suggesting need for continued activity of a β-adrenergic system throughout that period of memory formation. The other group (propranolol and Sotalol) apparently affected two processes, the first occurring at or about the time of training and the other acting briefly at about 25 min after training. The two groups also differed in the pattern of loss of memory that they induced. The Sotalol group produced delayed loss beginning at 40, and complete at 60 min after training; this time of loss was independent of time of injection and dosage. It is produced in the chick by a wide variety of amnestic agents [but not all: review, (3)], and appears to be associated with a sharply timed event in memory formation at about 50 min after training. The Timolol group, in contrast, produced loss soon after injection. The differences between the effects of these two categories of β-antagonist cannot be explained by simple pharmacokinetic differences (25). A number of pharmacodynamic

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hypotheses have been proposed to explain these differences and are discussed in more detail elsewhere (24,26).

Here, we investigate one of these hypotheses: that one, or both of the groups of β-antagonists also have effects at 5-HT sites. It is known that in slices of cortex from the rat brain (−)propranolol has antagonistic properties at 5-HT autoreceptors (18). This has also been demonstrated for other nonselective β-antagonists (19,20). 5-HT autoreceptors, mediating negative feedback regulation of the release of 5-HT, are located on 5-HT axon terminals in the CNS (7). The autoreceptors present in cerebral cortex, cerebellum, and hippocampus of the rat have been characterised pharmacologically and appear to belong to the 5-HT_{1B} subtype of 5-HT receptor (6). It was a possibility, therefore, that antagonism by one or both groups of β-antagonists at a similar autoreceptor in the chick might impair memory formation through consequent elevation of 5-HT levels. Amnesia is produced in the chick, using the task employed here, by 5-HT injected intracranially immediately before or immediately after training (12). A substantial body of evidence indicates that 5-HT plays an important role in the mediation of processes underlying learning and memory. Work in the rat has indicated that central elevation of 5-HT levels can induce specific deficits in the acquisition of both active and passive avoidance tasks [for a review, see (21)]. Recent results suggest that 5-HT exerts a negative influence on pathways crucial for memory formation, particularly within the hippocampus (2). Here, we investigate the possibility that similar 5-HT processes also operate in the chick and go some way towards explaining the heterogeneous effects of β-adrenergic antagonists on the CNS.

A brief summary of our findings has been presented elsewhere (24).

EXPERIMENT 1: THE EFFECTS OF ADMINISTRATION OF 21-009 BEFORE TRAINING

Method

Male Warren sex-link chicks (mass 50 g) were housed in pairs (to avoid stress due to isolation) on arrival from the hatchery. Cages were 18 by 25 by 20 cm, painted a matt grey inside and illuminated from above by a 25 W 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 was that employed in earlier chick studies (4). It began with a pretraining experience, in which first a small white bead (3 mm diametyer) and then a red and a blue bead (6 mm diameter) were presented to the chicks. Presentation of each bead lasted 10 s; presentations were separated by 3 min. The pair of birds often fixated the bead simultaneously; 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. This presentation 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, and upward tilt of the bill). This typically occurred within 5 s. 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 s with an interval of 5 min. Retention was measured by the number of pecks made at the red bead during the 10-s test period, and these numbers were used in analysis by ANOVA. All treatment groups were initially 20 birds; birds failing to peck at training or failing to show signs of aversion after pecking were excluded, reducing group sizes to a minimum of 16. In this experiment 21-009 (Sandoz), an agent with high affinity for 5-HT_{1B} receptors and some for β-receptors (14) but no affinity for any other class of 5-HT receptor (22), or saline was injected intracranially at a range of times from 60 min before to 25 min after training, at a dose of 15 μg/hemisphere in a 10 μml injection, using an automatic dispensing syringe. This was a free-hand injection, through the soft unossified skull roof to a depth of 2 mm in the center of each hemisphere; depth was controlled by a stop on the needle. The whole procedure is shorter and produces less and briefer disturbance of behavior than (for example) SC injection. The standard volume used for intracranial injection with chicks is 10 μl. Retention of drug within the injected hemisphere is higher than with smaller volumes (23), both absolutely and in proportion of total dose; unilateral injection gives effects specific to the injected hemisphere (4).

All experiments were performed blind, with treatment groups randomized between individual cages.

Results

When 21-009 was given 60 min before training (Fig. 1), it caused a marked and highly significant decrease in the number of birds avoiding the red bead at test. An ANOVA performed on the data presented in Fig. 1 revealed significant effects, $F(6, 168) = 3.598$, $p < 0.002$, with main effects associated with drug, $F(1, 168) = 8.127$, $p < 0.005$, and time of injection, $F(5, 168) = 2.736$, $p < 0.02$, proving to be significant.

Paired comparisons made between animals treated with 21-009 and saline showed significant effects when injections were made 60 min before training, $t_{32} = 2.208$, $p < 0.03$, and 30 min before training, $t_{35} = 2.04$, $p < 0.05$; at 5 min before training there was a suggestive difference, $t_{31} = 1.97$, $p = 0.057$.

Discussion

The most striking feature of the results is the contrast between retention when injections are given before training and

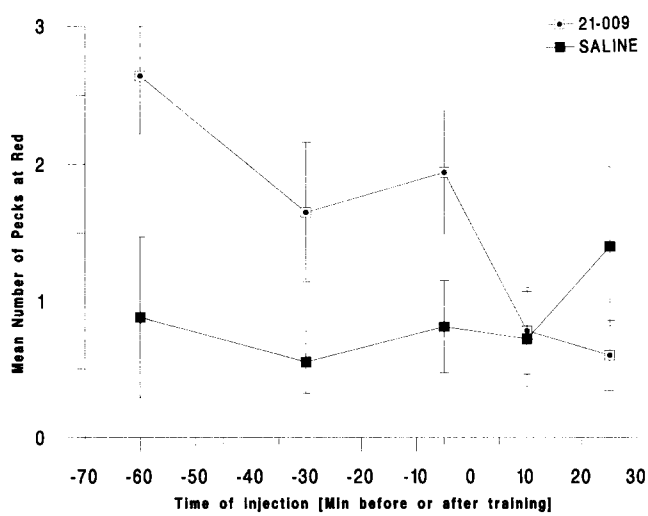

FIG. 1. The mean number of pecks (SEM) made towards the red bead at test are shown for groups of birds injected intracranially with 5-HT_{1B} antagonist (15 μg/hemisphere) or saline (154 mM NaCl) at the times shown. All tests were performed 3 h after training.

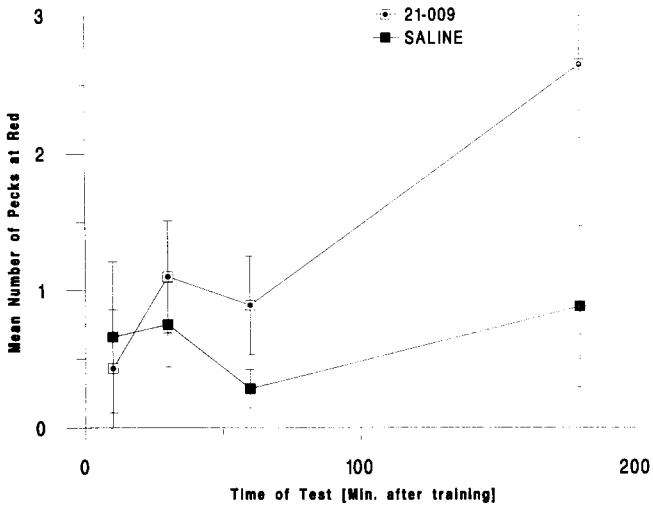

FIG. 2. The mean number of pecks (SEM) made towards the red bead at test are shown for groups of birds injected intracranially with 5-HT_{1B} antagonist (15 μg/hemisphere) or saline (154 mM NaCl). Injections were made 60 min before training and retention was tested 10, 30, 60, and 180 min after training.

those given after training. Although the difference between experimental and control groups just falls short of significance for the point 5 min before training, the difference is very like that at 30 min before, whereas injection at 10 and 25 min after training both show the same absence of any effect. The timing of the change in the curve strongly suggests action of 21-009 at (or just after) the time of training, rather than at test.

EXPERIMENT 2: TIME COURSE OF LOSS OF RETENTION AFTER 21-009

Clearly, any agent that acts only when given before training may be having nonspecific effects, which may interfere with the learning of the task. It was, therefore, important to establish whether the animals showed any evidence of learning the task and if so, for how long. A first question in comparing the effects of 21-009 and those of the β-antagonists, which have already been discussed, is whether 21-009 also shows delayed loss of memory. It should also be noted that presence of memory for some time after learning makes it unlikely that an agent is acting to prevent learning by nonspecific disturbance of behavior. The procedures were the same as in Experiment 1, except for injection times (all birds were injected 60 min before training) and for the timing of the retention tests, which were given at 10, 30, 60, and 180 min after training.

Results

Memory was initially good, and continued so until 60 min. Amnesia developed at some time thereafter, and was marked 180 min after training (Fig. 2). An ANOVA performed on the data presented in Fig. 2 revealed significant effects, $F(4, 118) = 3.880$, $p < 0.005$, with the main effects associated with drug, $F(1, 118) = 5.667$, $p < 0.02$, and time, $F(3, 118) = 3.213$, $p < 0.03$, proving to be significant. Pair-wise comparison indicated that the drug produces a significant deficit in retention compared to the saline control, when retrieval is measured at 180 min ($p < 0.03$) but not before.

Discussion

Learning clearly occurs, despite the actions of 21-009, at or around the time of training. Delayed loss of memory is strongly suggested by the results obtained here. It resembles that which we have described for β-antagonists like Sotalol, except that here 21-009 did not result in loss until after 60 min, whereas the Sotalol group produce loss that is complete by 55 min. Further work is required to see whether this is a genuine difference or not.

EXPERIMENT 3: POSSIBLE EFFECTS OF 21-009 AT TEST

Although the results in Experiment 1 strongly suggest action of 21-009 at about the time of training but not thereafter, we wished to examine further the possibility of action at test. To do this, 21-009 was given 5 min before test. Procedures and dose were as in previous experiments, in particular, the interval between training and test was, again, 180 min.

Results and Discussion

Injection of 21-009 5 min before test had no effect on behavior (Fig. 3), further confirming that the drug has little or no direct effect on behavior at test. These results are quite different from those obtained when injections are made 5 min before training. The possibility remains that 21-009 does act at test, but only after a very long period of central action. On such a hypothesis, Experiment 1 would be interpreted as showing action at test beginning about 185 min after injection. Experiment 2 would show no action between 65 and 120 min after injection, but action beginning by 210 min. Experiment 3 would exclude action very soon after injection. We think this explanation an unlikely one; it makes the relationship between the point of change in the results and training in Experiment 1 a coincidence and it fails to explain the apparent difference between the effects of injection at 5 min before training and 5 min before test. It, thus, seems probable that

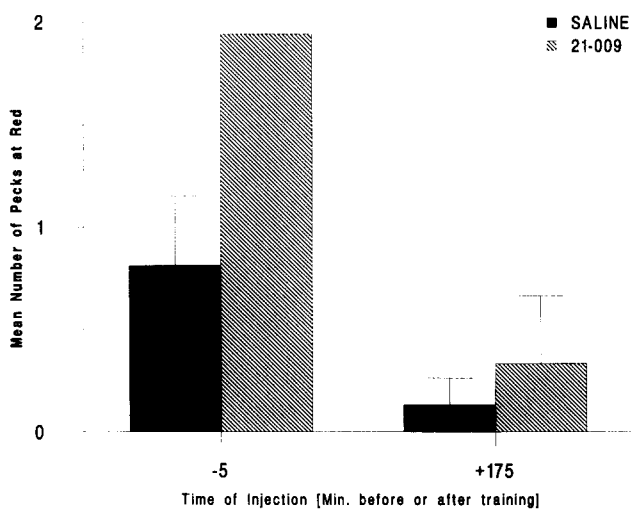

FIG. 3. The mean number of pecks (SEM) made towards the red bead at test are shown for groups of birds injected intracranially with 5-HT_{1B} antagonist (15 μg/hemisphere) or saline (154 mM NaCl). Injections were made either 5 min before training or 5 min before test, which was 180 min after training. The values for 5 min before training are repeated from Fig. 1 (where the full SEM is shown).

21-009 acts on mechanisms operating at or about the time of learning.

EXPERIMENT 4: THE EFFECTS OF 5-HT$_2$ AND 5-HT$_3$ BLOCKADE ON MEMORY FORMATION

Despite the reported lack of action of 21-009 at the 5-HT$_2$ and 5-HT$_3$ receptors, a direct test of the effects of blockage of postsynaptic 5-HT receptors seemed necessary.

Method

This was done by comparing the effects of Ketanserin (selective 5-HT$_2$ antagonist Janssen) and ICS 205-930 (selective 5-HT$_3$ antagonist, Sandoz), both injected 30 min before training at the same dose (15 μg/hemisphere in 10 μl saline) as was used for 21-009, together with saline controls.

Results and Discussion

Neither Ketanserin nor ICS 205-90 differ significantly from saline-injected controls (Fig. 4) when they are injected 30 min before training. In contrast, Altman and Normile (1) found amnesia to begin to appear in mice given Ketanserin before, but not after, training at the dose 0.56 mg/kg, a dose certainly exceeded here. The discrepancy may be due to species or task differences; clearly, a wider range of doses needs to be examined before concluding that blockade of 5-HT$_2$ or 5-HT$_3$ receptors has no effect on learning and/or memory formation in the chick. However, the issue here is that potent postsynaptic serotonergic antagonists have no effects in the chick at a dose at which 21-009 is effective. This, together with the reported (22) lack of action of 21-009 at 5-HT receptors other than 5-HT$_{1b}$, makes it very unlikely that 21-009 owes any of its amnestic action in the chick to direct effects of 21-009 at postsynaptic 5-HT sites.

GENERAL DISCUSSION

The results presented here are consistent with the widely held view that elevated 5-HT levels in the CNS produce deep and sustained amnesia in a variety of experimental tasks designed to measure memory formation. A number of postsynaptic routes of action have been postulated to explain the effects of modulating 5-HT levels in the rat; for example, direct effects on 5-HT$_2$ systems (21) and interactions with cholinergic systems (2). We are not here concerned with the routes by which elevated 5-HT may be amnestic. Instead, having shown that this appears to be the case for the chick as for various mammals, we wish to argue that resemblances between the amnestic effects of 21-009 and of some β-adrenergic antagonists suggest that the latter may act via partial blockade of 5-HT$_{1B}$ autoreceptors. A diverse range of amnestic agents resemble 21-009 in that they act at or just after learning to bring about delayed loss of memory in the chick. Such agents include protein synthesis inhibitors (11), many nonessential amino acids (12), and anti-Thy-I (17), as well as the β-adrenergic antagonists Sotalol and propranolol (25). Loss is typically completed by 55 min rather than at some time after 60 min, which appears to be the case when 21-009 is administered.

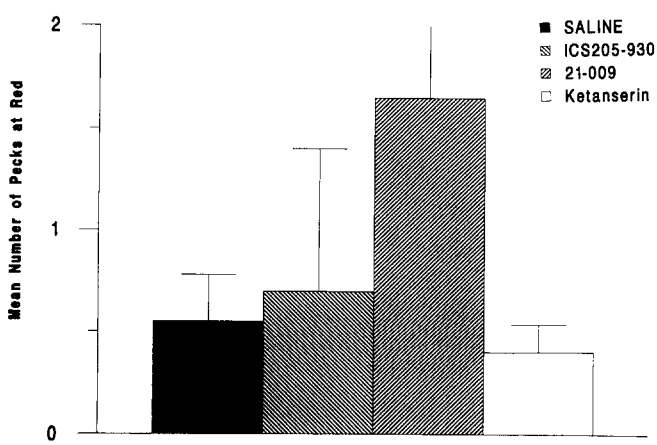

FIG. 4. The mean number of pecks (SEM) made towards the red bead at test are shown for groups of birds injected intracranially with a 5-HT$_2$ (ketanserin), or a 5-HT$_3$ (ICS205-930) antagonist (15 μg/hemisphere), or saline (154 mM NaCl), 30 min before training. Tests were performed 3 h after training. The value for 21-009 is taken from Fig. 1 (−30 min), where the full SEM is shown.

A second difference between 21-009 and other agents that produce delayed loss, is that (with the exception of nonessential amino acids, which resemble 21-009 in this regard) the other agents are effective up to about 20 min after learning. Nevertheless, 21-009 clearly has a pattern of effects that are broadly similar to those of Sotalol and propranolol, and quite different from the pattern associated with the other group of β-antagonists (Nadolol and Timolol), which are effective considerably later than 20 min and which cause rapid rather than delayed memory loss (25).

Blockade of 5-HT$_{1B}$ receptors is likely to produce elevated levels of 5-HT, because they are presynaptic autoreceptors. One route by which elevated 5-HT might act is by rapid downregulation of β-adrenoceptors: this has been shown to result from elevation of 5-HT levels due to subacute administration of selective 5-HT uptake inhibitors like Sertraline (16). At the same time agonist, but not antagonist affinity of β-adrenoceptors, is decreased. It is, therefore, interesting that propranolol acts as an antagonist at 5-HT autoreceptors in rat cortex (18). The propranolol/Sotalol group of β-antagonists may, thus, produce their amnestic effects by a combination of action through elevation of 5-HT levels and antagonistic action at those β-adrenergic receptors which have not been downregulated. Such a combination would be expected to produce unusually complete and specific depression of noradrenergic function.

The differences in effect of 21-009 and of Sotalol or propranolol might, on this argument, be due to moderate depression of noradrenergic function by 21-009, as against profound depression by the latter agents. Propranolol has 10–50 times higher affinity for β- than for 5-HT$_{1B}$ sites, whereas 21-009 shows the reverse pattern (14). It remains to be seen why the other group of β-antagonists (Nadolol and Timolol) produce such a different pattern of amnestic effects. Here, too, it is possible that direct antagonism of β-receptors is not the only route of action.

It should be noted that the three groups of agents, which are likely to have an important part of their action through depression of noradrenergic systems, fall into a series. The later after training that administration is effective, the shorter the time between administration and memory loss. Thus, the Timolol-Nadolol group act up to at least 40 min after training and are followed by rapid loss, when given 15 min after training (24). At the other end of the scale, 21-009 is effective only at (or perhaps just after) training and may cause loss at a time later than that associated with the Sotalol group.

Delayed effects of amnestic agents are of great interest, particularly when they have such standard timing as has been demonstrated in the chick. They strongly suggest that two routes are involved in memory formation and that disturbance in one can result at subsequent processing events in delayed action on the other which is used to determine response at recall tests. The evidence (above) that the weaker the initial action of an agent, the greater the delay before amnesia appears, can be explained by a longer period of interaction being required with a less profound initial disturbance. Andrew has argued (3) that bilateral memory formation involving both of the hemispheres provides the most likely candidate for two parallel routes. Antagonists to 5-HT_{1B} receptors appear to be very promising agents for the further study of the nature of the processes leading to delayed loss, and of the involvement of noradrenergic systems and their possible modulation by 5-HT in learning and memory formation.

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