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Short communication

Enhancement of memory consolidation in chicks by β_3-adrenoceptor agonists

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Abstract

The effects of intracranial injection of three β_3-adrenoceptor agonists, sodium-4-[-2[-2-hydroxy-2-(-3-chloro-phenyl)ethylamino] propyl]phenoxyacetate (BRL 37344), 2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl)1*H*-imidazole-2-yl)-phenoxy)-propyl)amino)ethoxy)-benzamide monomethane sulfonate) (±)-CGP12177A) and the pro-drug *RS-N*-(7-carbethoxymethoxyl 1,2,3,4-tetrahydronaphth-2-yl)-2 hydroxy 2-(3-chlorophenyl)ethanamine (SR58611A), were examined on reinforcement of memory in day-old chicks. BRL37344 and CGP12177 facilitated memory, whereas SR58611A had no effect. The dose–response relationships of the β_3-adrenoceptor agonists were challenged with the selective β_3-adrenoceptor antagonist 3-(2-ethylphenoxy)-1-[(1*S*)-1,2,3,4-tetrahydronapth-1-ylamino]-2*S*-2-propanol oxalate (SR59230A) or the β_2-adrenoceptor antagonist (−)propranolol. BRL 37344 appeared to act predominantly at β_3-adrenoceptors at low doses and at β_2-adrenoceptors at higher doses. Facilitation of labile into long-term storage by β_3-adrenoceptor agonists appears to be a class action of these drugs.

Keywords: β_3-Adrenoceptor; Memory; Consolidation; Memory, labile; Passive avoidance learning; Adrenoceptor; (Chick)

1. Introduction

Intracerebral injection of noradrenaline facilitates memory formation in the chick (Gibbs and Summers, 2000) and several studies in rodents have attributed this action of noradrenaline to an action on a number of adrenoceptor subtypes (e.g. Hatfield and McGaugh, 1999; Liang et al., 1986; Ferry et al., 1999). In the rat, injections of noradrenaline into the hippocampus, entorhinal regions (Izquierdo et al., 1998) and amygdala (Liang et al., 1986, 1990; Hatfield and McGaugh, 1999) enhance memory formation. Although peripheral administration of noradrenaline in the rat is unlikely to act directly in the brain as it does not cross the blood–brain barrier, the effect of intracerebrally administered noradrenaline has been attributed to an action on β_2-adrenoceptors (Ferry and McGaugh, 1999). However, in the chick with an undeveloped blood–brain barrier, subcutaneous (Crowe et al., 1990; Gibbs, 1991) or intracranial (Gibbs and Summers, 2000) injection of noradrenaline immediately after training results in consolidation of memory, where a labile memory trace lasting 30 min is changed into a permanent one. It has been proposed that the role of intracerebral noradrenaline in vivo in promoting memory consolidation is due to activation of β_1- and/or β_2-adrenoceptors since memory loss is produced by administration of the β_{1+2}-adrenoceptor antagonists sotalol and propranolol (Stephenson and Andrew, 1981; Crowe et al., 1991). However, we have shown recently, using β_1-, β_2- and β_3-subtype selective agonists and antagonists, that both β_2- and β_3-adrenoceptors, but not β_1-adrenoceptors, have an important role in the consolidation of memory mediated by effects in the hyperstriatum ventrale. The effects on memory consolidation of specific agonists at these receptors can be discriminated both pharmacologically and temporally (Gibbs and Summers, 2000).

The agonist used in these experiments CL316243 (disodium (*R,R*)-5-[2-[[2-3-Chlorophenyl)-2-hydroxyethyl]-amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate) is highly selective for β_3- in comparison to β_1- or β_2-adrenoceptors (Dolan et al., 1994), but little is known of its action at other G-protein coupled receptors. In order to establish whether the effect on memory is confined to

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CL316243 or is a class action of β_3-adrenoceptor agonists, we have compared the effects of CL316243 with the actions of 3 other known β_3-adrenoceptor agonists-sodium-4-[-2[-2-hydroxy-2-(-3-chloro-phenyl) ethylamino] propyl] phenoxyacetate (BRL 37344); 2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl) 1*H*-imidazole-2-yl)-phenoxy)propyl)amino) ethoxy)-benzamide monomethane sulfonate ((±)-CGP 12177A) and the pro-drug *RS-N*-(7-carbethoxymethoxyl 1,2,3,4-tetrahydronaphth-2-yl)-2 hydroxy 2-(3-chlorophenyl)ethanamine (SR58611).

2. Materials and methods

In the one trial discriminated avoidance learning paradigm, chicks discriminate between two different coloured beads, and if they remember, avoid one of the colours but continue pecking at the other. Day-old chicks were trained on a weakly reinforced stimulus—a red bead dipped in 20% anthranilate, which is mildly aversive. Untreated, or saline-injected chicks, remember this aversive association on tests made up to 30 min after training, but in tests made later than, this there is retention of progressively less and less memory until at 50 min after training, the chicks peck the red and the blue bead almost equally (Crowe et al., 1989; Gibbs, 1991; Gibbs and Summers, 2000). Memory is scored as a discrimination ratio, i.e. the ratio of the number of pecks to the blue bead in the 10-s retention test to the total number of pecks at the red and the blue bead in each of the 10-s tests. When a chick remembers the association, the discrimination ratio approaches 1.0 but failure to remember causes it to approach 0.5.

In the first series of experiments, where the action of the agonist is challenged by either the β_2- or the β_3-adrenoceptor antagonist, the agonists are injected into the hyperstriatum ventrale 20 min after training and antagonists are given systemically 5 min after training, 15 min before the agonist. The chicks are tested 120 min after training to see whether the respective antagonists have prevented the action of the agonist promoting memory consolidation.

In the second series of experiments, two doses of BRL37344 were injected intracerebrally at selected times after training on 20% anthranilate. The two doses were 10 pmol and 1 nmol and were chosen on the basis of the ability of SR59230A and (−)-propranolol to prevent the enhancement of memory formation. Subsequently, the level of memory retention was investigated (in separate groups of chicks) at different times after training, with injection of the 10 pmol or 1 nmol doses 2.5 min after training.

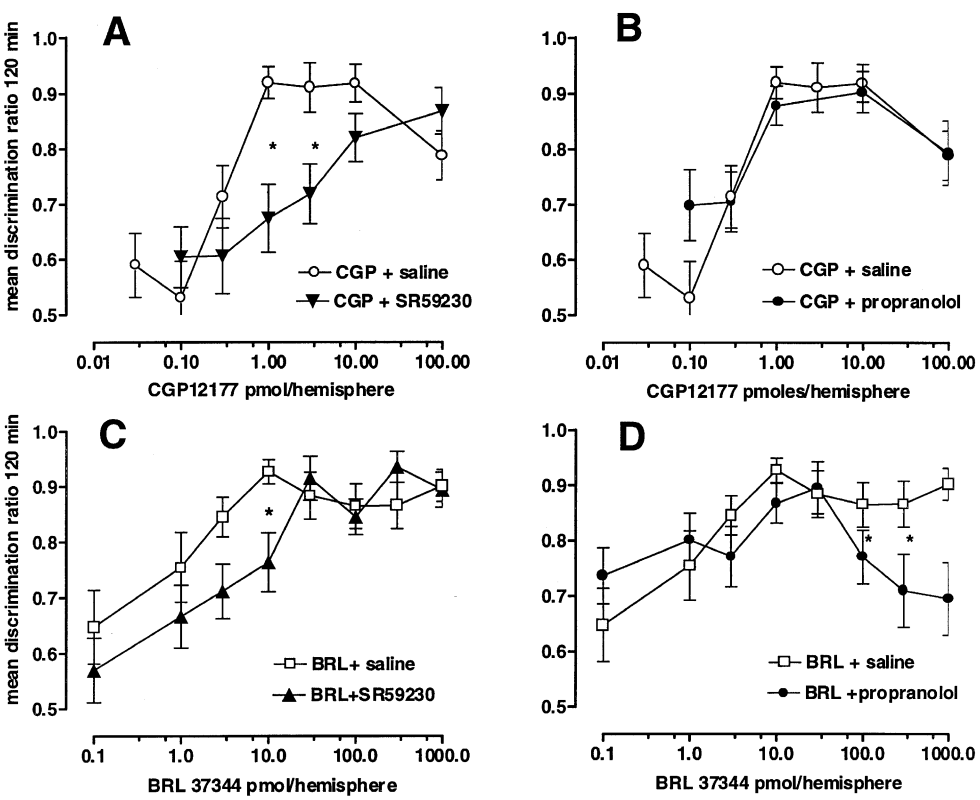

Fig. 1. Dose–response relationship for CGP12177 (A, B) or BRL37344 (C, D) in the presence of saline (open symbols), SR59230A (A, C) or (−)-propranolol (B, D). β_3-Adrenoceptor agonists were administered by intracerebral injection into each hemisphere 20 min after training on 20% anthranilate. The β_3-adrenoceptor antagonist SR59230A (~250 pmol/kg), β_{1+2}-adrenoceptor antagonist propranolol (~250 nmol/kg), or saline were administered by subcutaneous injection 5 min after training. SR59230A shifted the dose–response curve for CGP12177 (A) and BRL 37344 (C) to the right, whereas propranolol had no effect (B, D). With propranolol, memory consolidation with high doses of BRL 37344 was inhibited (D).

In order for the agonists to have an effect on memory, they are injected within 30 min, i.e. during the lifetime of the labile memory. In experiments examining the effect of an agonist on memory (Gibbs and Summers, 2000), the agonist is given intracerebrally 20 min after training BRL37344, CGP12177A and SR 58611 were administered by bilateral injections (10 μl) into the intermediate hyperstriatum ventrale of the forebrain using a Hamilton Repeating Dispenser syringe and a 27-gauge needle fitted with a sleeve to limit the depth of injection to 3.5 mm. The injection sites were 2–3 mm on either side of the midline and 3–4 mm forward of the tegmentum, into a sensory motor integration or cortical area (Reiner et al., 1984). SR59230A and (−)-propranolol, were administered by subcutaneous injection (100 μl) at 5 min after training at doses of ~250 pmol/kg or ~250 nmol/kg, respectively. Although these antagonists will produce memory deficits, the doses employed in this study were not high enough to produce amnesia. The chicks were tested 120 min after training. The injection volumes (10 μl), although large for a mammalian brain where injection would be expected to produce damage because of the bony skull and compact nuclei, produce no damage in the chick where there are large extracellular spaces and an unossified skull. Controls that receive a saline injection show no deleterious effect on behaviour. All procedures were in accordance with the guidelines of the Monash University Animal Ethics Committee and comply with the 1996 guidelines of the National Health and Medical Research Council of Australia code of practice for the care and use of animals for scientific purposes.

The β_3-adrenoceptor agonists were administered at a range of doses-BRL37344 (0.1 pmol–1.0 nmol/hemisphere); CGP12177 (0.01–100 pmol/hemisphere) and SR58611A (0.001–1.0 nmol/hemisphere). The authors thank the following companies and individuals for gifts of: BRL37344 (sodium-4-[-2[-2-hydroxy-2-(-3-chloro-phenyl) ethylamino] propyl] phenoxyacetate) (Dr. M.A. Cawthorne, Smith Kline Beecham, Great Burgh, Epsom, UK); SR58611A (*RS-N*-(7-carbethoxymethoxyl 1,2,3,4-tetrahydronaphth-2-yl)-2 hydroxy 2-(3-chlorophenyl)ethanamine); SR59230A (3-(2-ethylphenoxy)-1-[(1*S*)-1,2,3,4-tetrahydronapth-1-ylamino]-2*S*-2-propanol oxalate) (Dr. Luciano Manara, SANOFI-MIDY Research Centre, Milan, Italy). Other chemicals were from commercial sources: (−)-propranolol (Sigma, St. Louis, MO, USA), (±)-CGP 12177A (2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl) 1*H*-imidazole-2-yl)-phenoxy)propyl)amino) ethoxy)-benzamide monomethane sulfonate) Research Biochemicals International.

3. Results

Administration of BRL37344 or CGP12177 promoted memory consolidation in a dose-dependent manner (pEC_{50} = 1.0 pmol ($F(7,300) = 11.15$; $P < 0.0001$) and 0.3 pmol ($F(5,215) = 10.34$; $P < 0.0001$), respectively). Dose–response curves for both BRL37344 and CGP12177 (Fig. 1A,C) were shifted to the right by prior administration of SR59230 (~250 pmol/kg, s.c.). A two-way ANOVA comparing the action of saline or SR59230 on BRL 37344-treated chicks revealed a significant drug effect

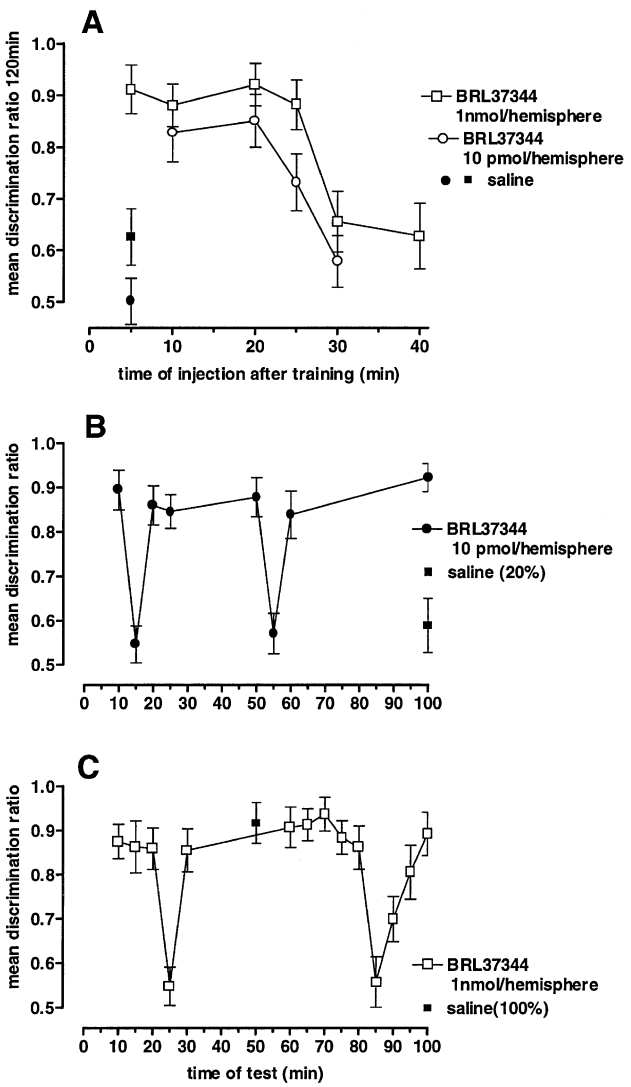

Fig. 2. (A) Injection of 10 pmol or 1 nmol/hemisphere of BRL37344 into the intermediate hyperstriatum ventrale of the chick brain at different times after training was effective in promoting consolidation of labile memory when given up to 25 min after training on 20% anthranilate but not when injected at 30 min or later. Injection of saline did not promote memory consolidation. (B) Promotion of memory consolidation by injection of 10 pmol/hemisphere of BRL 37344 2.5 min after training on 20% anthranilate. The timing of the stages is the same as that seen with strongly reinforced training and is typical of an action mediated through β_3-adrenoceptor (Gibbs and Summers, 2000). Saline control chicks trained on 20% anthranilate. (C) Extension of STM and intermediate memory following BRL37344 1 nmol/hemisphere. Chicks given weakly reinforced training. For comparison, saline control chicks were given strongly reinforced training with 100% anthranliate. This pattern is typical of an effect mediated through β_2-adrenoceptor (Gibbs and Summers, 2000).

($F(1,300) = 4.87$; $P = 0.028$); however, the drug × dose interaction was not significant.

For CGP 12177A and SR59230, there was a significant drug × dose interaction ($F(5,215) = 3.66$; $P = 0.003$) with a significant drug effect ($F(1,215) = 7.47$; $P = 0.007$). The dose–response curve for CGP12177 was not shifted by pre-administration of propranolol (~250 nmol/kg, s.c.) ($F(2,104) = 2.25$; $P = 0.110$) (Fig. 1B) but the action of the higher doses of BRL37344 was prevented by propranolol ($F(7,285) = 2.11$; $P = 0.042$) (Fig. 1D).

Administration of four dose levels of SR58611 had no effect on memory consolidation, with the discrimination ratios being not significantly different from those of the saline control (data not shown—dose range 0.001–1.0 nmol/hemisphere).

The two doses of BRL37344 (1 nmol and 10 pmol/hemisphere) whose actions were prevented by (−)-propranolol and SR59230, respectively, were both effective in promoting memory consolidation when administered at similar times (Fig. 2A). Injected between 5 and 25 min after training, the discrimination ratios after both doses of BRL37344 were significantly higher that the discrimination ratio for chicks injected with saline 10 min after training ($F(4,85) = 8.028$; $P < 0.001$ and $F(6,118) = 7.628$; $P < 0.001$, respectively). When injected 30 or 40 min after training, there was no effect, and the results were not significantly different from those seen when saline was injected.

As predicted, the time course of memory formation following injection of the 10 pmol/hemisphere dose of BRL37344 followed the pattern seen with controls given strongly reinforced training, and in addition the duration of short-term and intermediate memory followed that seen with the β_3-adrenoceptor agonist CL316243 (Gibbs and Summers, 2000) (Fig. 2B). As what happens with the injection of the β_2-adrenoceptor agonist zinterol, the duration of both short-term and intermediate memory was increased following injection of 1 nmol/hemisphere of BRL37344 (Fig. 2C). With both doses of BRL37344, there were two times of test when there was a group of chicks with a significantly lower discrimination ratio than at other times—15 and 55 min after training for 10 pmol and 25, 85 to 90 min after training for 1 nmol ($F(8,156) = 11.720$; $P < 0.001$ and $F(15,262)$; $P < 0.001$, respectively).

4. Discussion

The pharmacological profile of the three β_3-adrenoceptor agonists used in the present studies differ. BRL37344 is a selective β_3-adrenoceptor agonist (Arch et al., 1984) with some β_2-adrenoceptor properties at high doses (Fraeyman et al., 1992). CGP12177 is a partial agonist at the β_3-adrenoceptor and a potent antagonist at both β_1 and β_2-adrenoceptors (Feve et al., 1991) and has also been proposed to act at a 'putative β_4-adrenoceptor' that is distinct from the β_3-adrenoceptor (Molenaar et al., 1997). CGP12177 produced a steeper dose–response curve than BRL37344, possibly because of its additional action as a β_2-adrenoceptor antagonist. SR58611 acts at β_3-adrenoceptors through an active metabolite formed by de-esterification. These esterases are found in the walls of the colon and for this reason SR58611A was originally introduced as a gut-specific β_3-adrenoceptor agonist (Bianchetti and Manara, 1990). The finding that SR58611 had no action in promoting memory consolidation may be explained if the chick brain lacks the appropriate esterases.

The actions of the different β_3-adrenoceptor agonists are interpreted in the framework of a three stage model of memory in the chick (Watts and Mark, 1971; Gibbs and Ng, 1977; Gibbs, 1991). In this model, there are three stages in memory processing that have precise meanings and are seen in both behavioural studies and studies with drug intervention (Gibbs, 1991). Various hormones can alter the duration of short-term and/or intermediate memory depending on the stage of memory acted on by the hormone (Gibbs et al., 1991; Gibbs and Ng, 1984). β_3-Adrenoceptor agonists in the weak reinforcement paradigm cause good memory retention 120 min after training. Stimulation of β_3-adrenoceptors appears to reinstate memory formation processes, and the duration of short-term and intermediate memory are the same as that seen after strongly reinforced training (Gibbs and Summers, 2000). In contrast, β_2-adrenoceptor agonists cause the duration of both short-term and intermediate memory to be prolonged.

Although we found that the β_3-adrenoceptor agonist CL316243 and the β_2-adrenoceptor agonist zinterol were both effective in enhancing consolidation when injected up to 20 or 25 min after training, a difference between the two adrenoceptor agonists was seen in the duration of short and intermediate memory (Gibbs and Summers, 2000). The duration of the two memory stages is defined by brief periods where there is poor recall of memory at 15 and 55 min after training (Gibbs and Ng, 1979; Gibbs, 1991). With CL316243, the duration of short and intermediate memories were the same as that observed with strongly reinforced training, whereas with zinterol, short-term memory was extended by 10 min and intermediate memory by 20 min. Further support for a dual action of BRL37344 comes from experiments in which memory was measured at different times after training following different doses of BRL37344. We have interpreted the effect of BRL37344 at a low dose (10 pmol) as mediated by β_3-adrenoceptors and that of a higher dose (1 nmol) as mediated by β_2-adrenoceptors (Fig. 1). If this is the case, then a difference in the memory time course after promotion of consolidation would be expected with the two different doses. When memory levels are measured in separate groups of chicks at different times after training, BRL37344 (10 pmol) given 2.5 min after training caused a pattern similar to CL316243; whereas BRL 37344 (1

nmol) produced an effect similar to zinterol (Fig. 2B). The duration of short-term memory, as measured by the timing of the dip in retention (Gibbs, 1991) was normal with BRL37344 (1 pmol) but was shifted to 25 min by BRL 37344 (1 nmol). Similarly, the dip between intermediate and long-term memory was at 55 min for BRL37344 (1 pmol), but shifted to 85 min by BRL37344 (1 nmol).

That these selective β_3-adrenoceptor agonists have an action in promoting memory consolidation suggests that the β_3-adrenoceptor in the chick is generically activated by the agonists. However, several questions remain including whether or not the molecular pharmacology of the chick brain adrenoceptor resembles that of the human or the rodent β_3-adrenoceptor (Summers et al., 1995) or is an avian receptor possibly the chick analogue of the turkey β_{4c}-adrenoceptor (Chen et al., 1994). There may also be differences in the patterns of memory formation between chicks and rodents. Models of memory formation in rodents, chicks and insects have described two stages (Izquierdo et al., 1999) or multiple stages (Xia et al., 1998; De Zazzo and Tully, 1995; Menzel et al., 1999; Rosenzweig et al., 1993). Whether these differences are results of the different learning paradigms used to determine them or are differences in the underlying mechanisms is not clear, and it is not known if working memory in the rat (Van der Staay, 1999) is equivalent to short- and/or intermediate memory in the chick.

In conclusion, the action of the β_3-adrenoceptor agonist CL316243 in promoting memory consolidation (Gibbs and Summers, 2000) was also displayed by two other β_3-adrenoceptor agonists-BRL37344 and CGP12177. These studies therefore provide further support for a role of β_3-adrenoceptors in promoting memory formation in the chick.

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