Neuroscience 131 (2005) 31–42

CONTRASTING ROLES FOR β_1, β_2 AND β_3-ADRENOCEPTORS IN MEMORY FORMATION IN THE CHICK

M. E. GIBBS* AND R. J. SUMMERS

Department of Pharmacology, Monash University, Wellington Road, Clayton, Victoria 3800, Australia

Abstract—Noradrenaline plays distinct roles in the modulation and consolidation of memory for one-trial, discriminated, avoidance learning in the chick. We have previously shown that activation of β_2-, β_3- and α_1-adrenoceptors (ARs) by injection into the multimodal forebrain association region (intermediate medial hyperstriatum ventrale [IMHV] or intermediate medial mesopallium [IMM]) is involved in the consolidation of memory 30 min after training and that activation of α_2-ARs in the caudate putamen plays a role in the reinforcement of memory leading to consolidation in the IMM (IMHV). In this paper we provide evidence that noradrenaline acts at β_1-ARs in the basal ganglia (lobus parolfactorius or medial striatum) in short-term memory processing immediately post-training and demonstrate inhibition of memory by selective AR antagonists at particular times in the sequential memory processing sequence after training. These results support separate roles for β_2- and β_3-ARs in memory consolidation. Our studies suggest that, as a consequence of the learning experience, noradrenaline acts in different brain regions and at different times in memory processing, to enhance memory through distinct populations of ARs.

Key words: discriminated avoidance learning, noradrenaline, short-term memory, intermediate memory, memory consolidation, memory enhancement.

Noradrenergic innervation in the forebrain is widespread, but the response to synaptically released noradrenaline is a function of the adrenoceptor (AR) subtypes present since the three major groups of ARs (α_1, α_2 and β) mediate distinctive actions via modulation of the various intracellular signaling pathways. An additional layer of complexity is added by the knowledge that ARs are found not only on neurones but also on astrocytes and endothelial cells. Consequently, the interpretation of the actions of noradrenaline on brain function will need to accommodate the multiple actions and locations of ARs. Noradrenaline in the chick and mammalian forebrain is found in nerve terminals emanating from cell bodies located in the locus coeruleus (LC), and noradrenaline release is related to arousal, attention as well as stress (Berridge and Waterhouse, 2003). Although the nerve fibers project widely from the LC, the monoaminergic system is well organized and, like other afferent systems in the cortex, shows anatomical specificity (Papadopoulos and Parnavelas, 1991; Berridge and Waterhouse, 2003; Cardin and Schmidt, 2004). Noradrenaline has long been known to have modulating effects, both beneficial and deleterious, on memory processing, but attempts to determine its action by destruction of the LC or by lesioning the connections between the LC and the forebrain have been inconclusive, probably because of compensatory mechanisms.

Using selective pharmacological agents we have shown distinct roles for different ARs in the consolidation of memory in the forebrain multimodal association area in the chick: the intermediate medial hyperstriatum ventrale (IMHV) now the intermediate medial mesopallium (IMM) and the caudate putamen or lobus parolfactorius (LPO) now medial striatum (MSt) of the basal ganglia. In a recent forum these areas have been renamed to a form that can be equated with the mammalian nomenclature (Reiner et al., 2004). The LPO has been renamed the MSt and the IMHV has been renamed as the IMM.

Stimulation of β_2- and β_3-ARs is sufficient to consolidate an early labile memory to a state where it becomes relatively permanent (Gibbs and Summers, 2000). This consolidation in IMM (IMHV) requires adrenergic signaling in the MSt (LPO), where stimulation of α_2-ARs 10–15 min post training reinforces the storage in IMM (IMHV; Gibbs and Summers, 2003).

Memory resulting from weakly reinforced training is labile, lasting up to 30 min and is not retained unless a subsequent event triggers consolidation into permanent storage. An increase in noradrenaline levels, achieved by central injection of noradrenaline will promote the consolidation of memory. This injection must be made during the lifetime of labile memory, immediately to 30 min after training: a later injection has no effect on subsequent memory formation.

The facilitatory action of noradrenaline injected into the IMM (IMHV) 20 min after learning has been attributed to activation of β_2- and β_3-ARs (Gibbs and Summers, 2000) and this effect can be antagonised by selective β_2- and β_3-AR antagonists, whereas α_2-AR antagonists have no effect. In the IMM (IMHV), low doses of noradrenaline activate β_3-ARs whereas higher doses activate β_2-ARs to promote consolidation. Higher doses of noradrenaline administered into the IMM (IMHV) activate α_1-ARs and inhibit memory

*Corresponding author. Tel: +61-3-9905-9410; fax: +61-3-9905-8192.
E-mail address: marie.gibbs@med.monash.edu.au (M. E. Gibbs).

Abbreviations: ANOVA, analysis of variance; AR, adrenoceptor; CGP20712A, (±)-2-hydroxy-5-(2-((2-hydroxy-3-(4-(1-methyl-4-(trifluoromethyl)-1*H*-imidazole-2-yl)-phenoxy)propyl)amino)ethoxy)-benzamide monomethane sulfonate; DR, discrimination ratio; hem, hemisphere; IMHV, intermediate medial hyperstriatum ventrale; IMM, intermediate medial mesopallium; ITM, intermediate memory; LC, locus coeruleus; LPO, lobus parolfactorius: MSt, medial striatum; SR59230A, 3-(2-ethylphenoxy)-1-[(1*S*)-1,2,3,4-tetrahydronapth-1-ylamino]-2*S*-2-propanol oxalate; s.c., subcutaneous; STM, short-term memory.

0306-4522/05$30.00+0.00
doi:10.1016/j.neuroscience.2004.10.036

consolidation. In the MSt (LPO), injection of noradrenaline 10 min after training facilitates memory consolidation in the IMM (IMHV) and this is attributed to an action on α_2-ARs. Central injection of the selective β_1-AR agonist (RO363) into the IMM (IMHV) failed to promote memory consolidation given 20 min post-training (Gibbs and Summers, 2000).

There is evidence from other studies concerning the importance of noradrenergic activation of β-ARs in the modulation of memory, in both chicks (Stephenson and Andrew, 1981; Davies and Payne, 1989; Crowe and Shaw, 1997) and in rats (Izquierdo et al., 1998; Ferry et al., 1999; Sullivan et al., 2000; Tuinstra et al., 2002; McIntyre et al., 2003). Noradrenaline has been reported to have the potential to alter memory following retrieval (Przybyslawski et al., 1999).

In this paper we report on the role of β_1-ARs and the action of selective β_1-AR agonists and antagonists on short-term memory (STM), and on the action of selective β_2- and β_3-AR antagonists inhibiting memory consolidation following strongly reinforced training. The time at which memory consolidation is susceptible to disruption will reflect the time at which these receptors are required in memory processing (see Discussion). We find that β_1-ARs play a role in MSt (LPO) during acquisition and STM, whereas β_3-ARs are important in the IMM (IMHV) during the first phase of intermediate memory (ITM). β_2-ARs in the IMM (IMHV) are necessary for the consolidation of ITM 30 min after training.

EXPERIMENTAL PROCEDURES

Learning paradigm

Up to 240 1- to 2-day old Black-Australorp×White Leghorn or Rhode Island Red×New Hampshire male chicks (body weight 34.98±0.58 g brain weight 0.84±0.01 g; forebrain 0.44±0.01 g) were delivered from a local poultry farm (Research Poultry, Pty Ltd, Victoria, Australia) on the morning of each experiment. It might be expected that the chicks could be stressed because of the relocation from the hatchery. Our source of chicks is located relatively close to the University and care is taken during delivery. We have kept chicks overnight and do not find any differences in the response to AR agonists, and we have also hatched in the laboratory and find no differences in the baseline performance of the chicks to weakly reinforced training. If there were problems with transportation stress this would be apparent in the level of memory seen with weakly reinforced training. The experimental conditions were described in detail elsewhere (Gibbs and Summers, 2002b). Briefly, chicks were housed in pairs, in groups of 16–20 and allowed 2–3 h to become familiar with their new environment, including the presentation of beads to peck. After two 10 s presentations (30 min apart) of a small (2 mm diam.) shiny metal bead on the end of a 20 cm stiff wire, presentations (5 min apart) were made of a red, then a blue glass bead (4 mm diam.) which had been dipped in water. These two presentations of red and blue bead reveal an almost equal preference for red and blue beads (e.g. mean discrimination ratio [DR] 0.52–0.55). For the training trial, commencing at least 30 min later, the chicks were presented with an identical red bead that had been dipped in either 100% or 20% methyl anthranilate in alcohol (Sigma Aldrich Co.) to provide strongly or weakly reinforced training respectively. The chicks were allowed 10 s to peck at the training bead and they generally did so in the first 1–2 s. Strongly reinforced training resulted in good memory on test 120 min later, whereas weakly reinforced training resulted in a labile memory that lasts only for approximately 30 min. In one experiment, chicks were trained with 5% anthranilate on the bead, resulting in memory lasting for only 10 min). Although there was obviously some memory retained in this paradigm using 5% anthranilate, multiple trials 20 min apart required at least three presentations before memory is detectable 120 min later (M. E. Gibbs and R. J. Summers, unpublished observations). Memory retention, at specified intervals after training, was measured as the DR: the number of pecks at the blue bead relative to the total number of pecks at the red and blue bead on the successive test trials of 10 s duration. The pecks are recorded on a handheld recording logger that is decoded by computer at the completion of the experiment.

When a chick remembers the aversive taste of the red bead, it avoids pecking the red bead and the DR approaches 1.0; when a chick does not remember the DR approaches 0.5, i.e. the chick pecks at red and blue beads equally. Chicks can give up to as many as 12 pecks on the blue bead. Individual DRs were obtained for each chick and the data are presented as mean and S.E.M. Chicks that did not peck the bead during the training trial, or showed no discrimination and avoided the blue bead on test were eliminated from the data analysis at the completion of the experiment. There could be many reasons why chicks avoid the blue bead, not necessarily related to memory; however, never more than a couple of chicks were eliminated for this reason. The number of chicks per group generally varied between 14 and 20.

Table 1. Selective noradrenergic subtype agonists and antagonists

AR-subtype	Agonist	Antagonist	Site of action
β_1-AR	RO363	CGP20712A	MSt (LPO)
β_2-AR	Zinterol	ICI118551	IMM (IMHV)
β_3-AR	CL316243	SR59230A	IMM (IMHV)
β_1-/β_2-AR		Propranolol	IMM (IMHV)/MSt (LPO)
α_2-AR	Oxymetazoline	Yohimbine	MSt (LPO)

Drugs and injections

The selective AR agonists and antagonists used in this report are outlined in Table 1. The authors thank the following companies and individuals for gifts of: SR59230A (3-(2-ethylphenoxy)-1-[(1*S*)-1,2,3,4-tetrahydronapth-1-ylamino]-2*S*-2-propanol oxalate; Dr Luciano Manara; SANOFI-MIDY S.p.A. Research Centre, Milan, Italy); (±)-CGP20712A ((±)-2-hydroxy-5-(2-((2-hydroxy-3-(4-(1-methyl-4-(trifluoromethyl)-1*H*-imidazole-2-yl)-phenoxy)propyl)amino)ethoxy)-benzamide monomethane sulfonate; Dr G. Anderson; Ciba-Geigy AG, Australia). Other chemicals were from commercial sources as indicated: (−)-propranolol, (−)-noradrenaline bitartrate, yohimbine hydrochloride (Sigma Chemical Company, St Louis, MO, USA), (±)-ICI 118551 (erythro-DL-1(7-methylindian-4-yloxy)-3-isopropylaminobutan-2-ol; Imperial Chemical Industries, Wilmslow, Cheshire, UK); RO 363 ((±)-1-(3,4-dimethoxy-phenethylamino)-3-(3,4-dihydroxyphenoxy)-2-propranol)-oxalate; Institute of Drug Technology, Boronia, Australia); L(+)-ascorbic acid (Merck, Frankfurt, Germany).

Doses are expressed as pmol or nmol per hemisphere (hem) for central injections or per chick for subcutaneous (s.c.) injections. All drugs were diluted in 0.9% physiological saline. The stock solution of noradrenaline (10 mM) was made up in ascorbic acid (0.125 mM) to prevent oxidation and the controls in these experiments received the appropriate maximum dilution of ascorbic acid in saline. Drugs were given centrally by direct bilateral injection (infusion over 2–3 s) either into the MSt (LPO; 5 μl/hem), IMM (IMHV; 5 or 10 μl/hem) using a Hamilton Repeating Dispenser syringe or by s.c. injection (100 μl/chick) into a fold of skin ventral to the sternum.