ELSEVIER

Pharmacology Biochemistry and Behavior 67 (2000) 355–362

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

The mitochondrial benzodiazepine receptor and avoidance learning in the day-old chick

Fiona M. Freeman, Ian G. Young*

Division of Biochemistry and Molecular Biology, John Curtin School of Medical Research, The Australian National University, Canberra ACT 0200, Australia

Received 5 November 1999; received in revised form 1 June 2000; accepted 6 July 2000

Abstract

The specific mitochondrial benzodiazepine receptor (MBR) agonist, FGIN 1–27, and antagonist, PK 11195, were used to investigate whether this receptor was involved in passive avoidance memory formation in the day-old chick. PK 11195 at a concentration of 1–10 μM was found to be amnesic when injected directly into the lobus parolfactorius (LPO) 5 h after training ($P < .01$). Unilateral injections of PK 11195 further showed that memory was only disrupted with injections into the right hemisphere ($P < .01$). Since the MBR is considered to be involved in the production of a neurosteroid that modulates GABAergic transmission, we injected bicuculline and muscimol, specific inhibitor and agonist, respectively, of the $GABA_A$ receptor, to see if either disrupted memory formation. The results of bilateral injections into the LPO at 5 h post-training indicated that enhanced GABAergic transmission was involved in memory formation since the inhibitor, bicuculline, caused amnesia ($P < .01$) and unilateral injections also showed that this effect was confined to the right hemisphere ($P < .05$). Since memory for passive avoidance learning is thought to involve both cytosolic and mitochondrial protein synthesis at this 5-h time point [Freeman FM, Young IG. Chloramphenicol-induced amnesia for passive avoidance training in the day-old chick. Neurobiol Learn Mem 1999;71:80–93.], we studied the effect of unilateral injections of chloramphenicol (CAP) and anisomycin (ANI) during this second wave of protein synthesis and found that CAP only disrupted memory when injected into the right LPO 5 h post-training ($P < .05$). This lateralization to the right hemisphere was also seen when ANI was injected 4 h post-training ($P < .05$) but at 5 h, only bilateral injections of ANI could disrupt memory ($P < .05$). The results suggest a role for mitochondria and the GABAergic system in the retention of passive avoidance learning in the day-old chick.

Keywords: $GABA_A$; Protein synthesis; DBI; Memory

1. Introduction

Benzodiazepine (BZD) acts directly at the $GABA_A$ receptor to modulate chloride gating by the neurotransmitter GABA [11]. Indirectly, BZDs can also regulate neurotransmitter release via modulation of neurosteroid synthesis in the mitochondria [33]. The mitochondrial benzodiazepine receptor (MBR) is located in the outer mitochondrial membrane [1]. Steroidogenesis begins with the conversion of cholesterol to pregnenolone, which occurs in the inner mitochondrial membrane. Pregnenolone then leaves the mitochondrion to undergo enzymatic transformation in the endoplasmic reticulum. In some tissues, pregnenolone is metabolized to steroid intermediates that return to the mitochondria for the last step in the synthetic pathway, ultimately leading to the production of mineralocorticoids and glucocorticoids. The diazepam-binding inhibitor (DBI) is an endogenous polypeptide that has the ability to displace diazepam from the BZD binding site on the $GABA_A$ receptor and the MBR [27]. Stimulation of the MBR by DBI causes the translocation of cholesterol from the outer to the inner mitochondrial membrane, where it is converted to pregnenolone by the enzyme cytochrome $P450_{ssc}$ [32]. Some pregnenolone derivatives positively modulate and others negatively modulate GABA action at the $GABA_A$ receptor [33,37].

In the present work, we have investigated the role of the MBR in long-term memory formation in the chick. One-trial passive avoidance training in the day-old chick is an attractive model to study long-term memory formation. This paradigm exploits the precocity of newly hatched chicks

* Corresponding author. Tel.: +61-2-6249-2439; fax: +61-2-6249-0415.

E-mail address: ian.young@anu.edu.au (I.G. Young).

0091-3057/00/$ – see front matter
PII: S0091-3057(00)00373-7

which explore their environment by pecking and rapidly learn to distinguish between edible and distasteful objects. If a chick is presented with a bead coated in the bitter-tasting substance, methylanthranilate (MeA), it will peck once, show a characteristic disgust response, and subsequently avoid a similar, but dry, bead presented later [9,23]. This paradigm has the advantage that, since it requires only a single brief training trial, the exact time of memory inception is known and hence, the sequence of events that occur during memory consolidation. Using this paradigm, Freeman et al. [21] have shown the existence of two distinct waves of protein synthesis. The first occurs up to 90 min post-training and the other between 4 and 5 h after training. Apart from protein synthesis, two phases of neuronal activity following training have also been demonstrated in the chick [57]. Electrophysiological studies have shown a dramatic increase of spontaneous high-frequency neuronal bursting [40]. Initially, this bursting activity is distributed between left and right intermediate medial hyperstriatum ventrale (IMHV), but within 4–7 h shifts to the right IMHV and to the lobus parolfactorius (LPO) — a mammalian basal ganglion homologue [24,25]. A series of lesion studies [26,44,45] has shown that the IMHVs are involved in the acquisition of memory but not its retention, whereas the LPOs are involved in retention and recall but not the acquisition of memory for the passive avoidance training. Studies using c-Fos and c-Jun as markers of neuronal activity have also demonstrated a biphasic pattern of activity, where the IMHV is first activated followed by the LPO [20,53]. These findings support the concept of two phases of neuronal activity with information being processed in one area of the brain (e.g. IMHV) before being redistributed to other brain regions (e.g. LPO).

FGIN 1–27 has been used to study the MBR since it binds selectively to this receptor and not to the $GABA_A$ receptor or any other neurotransmitter receptors. It has also been shown to potently stimulate steroid biosynthesis in isolated glial cells and in brain mitochondrial preparations [50,51]. FGIN 1–27 stimulates pregnenolone synthesis in the rat brain and this is blocked by the selective MBR antagonist, PK 11195 [2,32,47]. Fear of novelty in rats is reduced by FGIN 1–27 and this fear was prevented by PK 11195 [51]. De Cunha et al. [15] have shown that memory for a stepdown inhibitory avoidance task involves the MBR and $GABA_A$ receptors. The endogenous ligand of the MBR, DBI, has been shown to impair performance in a working memory task [29]. In addition, the level of DBI has been found to be decreased in patients with Alzheimer's disease [19]. To study the role of the MBR in memory formation in the chick, we have used FGIN 1–27 and PK 11195.

Autoradiographical and biochemical studies have indicated the presence of GABA receptors in the chick brain, with particularly high receptor density in the IMHV and LPO [58,59]. Martijena and Arce [38] demonstrated a transient increase in the number of $GABA_A$ receptors in the chick forebrain just after passive avoidance training. Behavioural pharmacology has shown that pre-training injections of BZD impair imprinting in chicks [61]. Stimulation of the GABA receptor by muscimol disrupts passive avoidance memory formation, whereas inhibition of the receptor by bicuculline facilitates memory for this task when injected prior to training [10]. Mineralo- and glucocorticosteroids have also been shown to affect passive avoidance learning in the chick [54]. Since the DBI has also been shown to be present in chicken [8], it seems likely that the MBR will also be involved in regulating neurosteroidogenesis in chick brain mitochondria. In this paper, we have investigated whether the MBR and $GABA_A$ receptors are involved in passive avoidance memory formation during the second wave of neuronal activity in the LPO. Since both cytosolic and mitochondrial protein syntheses are thought to be involved in events at this 5-h time point [22], we have studied the effects of unilateral injections of inhibitors of both types of protein synthesis to try and learn more of the processes involved in memory formation and particularly of the role of mitochondria.

2. Methods

2.1. Preparation and injection of drugs

FGIN 1–27 and PK 11195 were both purchased from Sigma and initially dissolved in a minimal amount of DMSO plus a few drops of Tween-20 and then made up to their final concentrations with saline (0.9% NaCl) — the final concentration of DMSO was less than 0.5%. Chloramphenicol (CAP) was dissolved in a minimum volume of ethanol and the solution adjusted to a final concentration of 7.4 mM (2.4 mg/ml) with saline. A 30 mM (8 mg/ml) solution of anisomycin (ANI) was prepared by dissolving the solid in a minimal quantity of 3 M HCl, after which the pH was adjusted to 7 by the addition of 3 M NaOH and the concentration of ANI was then adjusted to 30 mM by the addition of saline. Muscimol and bicuculline were obtained from Sigma. Bicuculline was initially dissolved in ethanol before being adjusted to working concentrations with saline. Muscimol was dissolved directly in saline. The vehicle control in each case was a saline solution of the same composition as the solution used to dissolve the drug. For simplicity, it is referred to as saline in the figures. Only freshly prepared solutions were used. Intracranial injections of 10 μl per hemisphere were made directly into the LPO. The site and depth of delivery were controlled by the use of a specially designed stereotaxic headholder and sleeved Hamilton syringe [18]. The accuracy of the injection apparatus was verified by injection of dye and subsequent microscopic analysis of tissue.

2.2. Animals and training procedures

White leghorn–black Australorp chicks (*Gallus domesticus*) of both sexes were obtained on the day of hatching. The day-old chicks were placed in pairs into $20 \times 25 \times 25$ cm^3 aluminium pens, each illuminated with a 25-W red light left undisturbed for 2 h at 28–30°C before being trained on the one-trial passive avoidance paradigm described by Lössner and Rose [36]. Briefly, birds were pretrained with three 10-s presentations of a small (2.5 mm diameter) white bead. Chicks which pecked at least twice out of the three pre-training trials (at least 80%) were then trained by a 10-s presentation of a chrome bead (4 mm diameter) dipped in MeA. Drugs or vehicle control were injected at 5 h post-training. The chicks were then left overnight with food and water ad libitum until 24 h post-training when they were tested for recall by a 20-s presentation of a dry chrome bead and scored as Peck or Avoid. Each experiment was repeated on different days and the data pooled to remove batch-specific effects. Each bird was trained and tested only once. After testing with the chrome bead, birds were presented with a white bead similar to that used for pretraining to test the birds' ability to discriminate between aversive (chrome bead) and non-aversive (white bead) stimuli. The numbers of chicks which avoided the subsequent presentation of the white bead were insignificant for either drug- or vehicle-injected birds, verifying the validity of the avoidance data.

In the first experiment, chicks received bilateral intracranial injections of 10 μl at 5 h post-training of the MBR receptor antagonist, PK 11195, at concentrations of 500, 100, 10, and 1 μM or vehicle ($n = 10$, 11, 22, 23, and 22, respectively). The MBR receptor agonist, FGIN 1–27, was injected at concentrations of 600, 300, 100, 10, and 1 μM with vehicle as control ($n = 16–18$) and recall was tested for after 24 h. To see if the effect due to PK 11195 was lateralized, PK 11195 was injected 5 h post-training at 10 μM directly into either the left or right LPO, with the contralateral LPO receiving vehicle. For comparison, animals received either PK 11195 or vehicle into both hemispheres ($n = 22–24$) and recall was tested after 24 h.

Since stimulation of the MBR is thought to increase the production of neurosteroids that act as neuromodulators [43] of especially the $GABA_A$ receptor, we used the specific agonist and inhibitor of the $GABA_A$ receptor, muscimol and bicuculline, respectively, to determine if this receptor was involved in long-term memory formation at 5 h post-training. Bilateral intracranial injections of bicuculline were made into the LPO 5 h post-training using concentrations of 100, 50, 25, 10, and 5 μM with vehicle as a control ($n = 31$, 15, 15, 20, 17, and 31, respectively). Muscimol was also similarly injected at concentrations of 50, 25, and 5 μM with a vehicle control ($n = 10$). Recall was tested at 24 h. To test whether the effect due to bicuculline was lateralized, direct unilateral intracranial injections of bicuculline (100 μM) were made into the right or left LPO at 5 h post-training, with the contralateral LPO receiving vehicle. For comparison, birds received either bicuculline or vehicle in both LPOs ($n = 17–18$) and recall was tested at 24 h.

In a third set of experiments that were designed to determine if the effect on memory due to inhibition of mitochondrial protein synthesis is lateralized at this 5-h time point, the inhibitor of mitochondrial protein synthesis, CAP, was injected 5 h post-training at 7.4 mM directly into either left or right LPO, with the contralateral LPO receiving

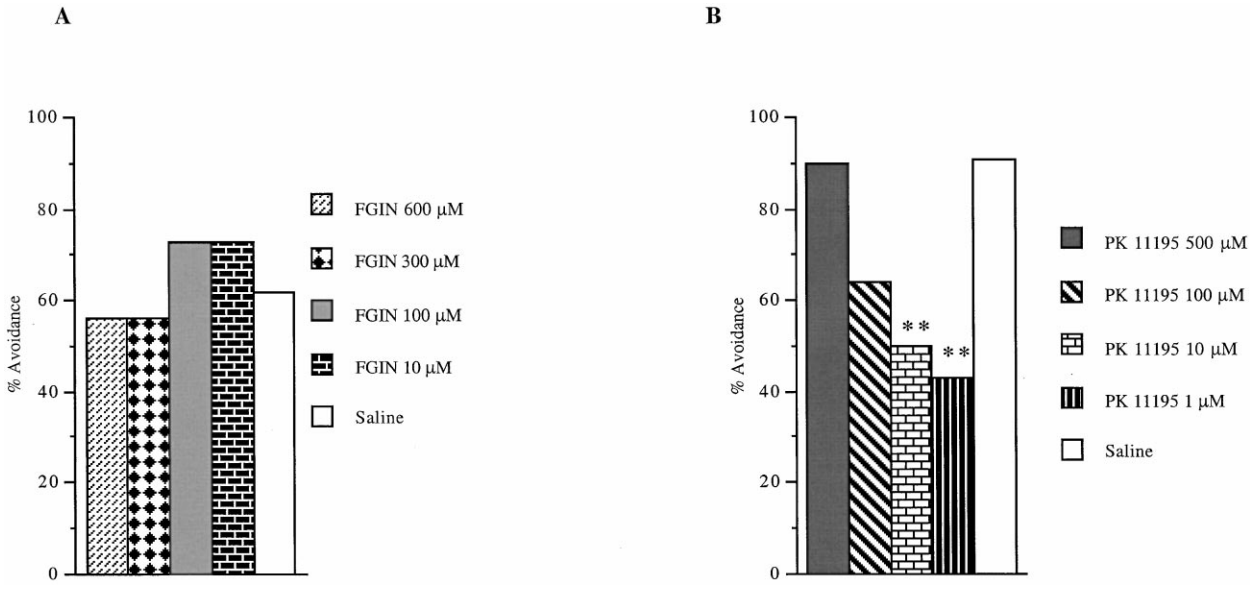

Fig. 1. Effect of bilateral intracranial injections (10 μl per hemisphere), directly into the LPO, at 5 h after passive avoidance training of (A) the MBR agonist, FGIN 1–27, or (B) the antagonist, PK 11195, at the indicated concentrations or vehicle. Numbers as described in Section 2. Retention was tested at 24 h. ** $P < .01$.

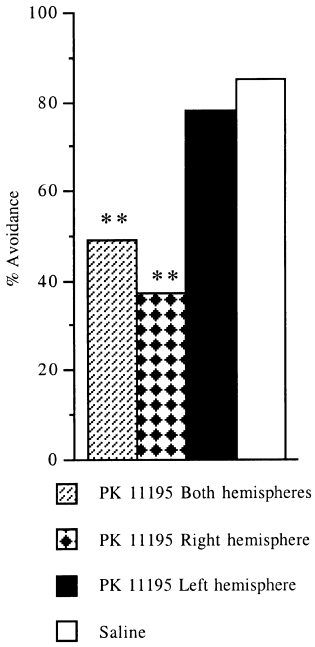

Fig. 2. Effect of unilateral intracranial injections directly into the LPO of 10 μM PK 11195 (10 μl per hemisphere) 5 h post-training. Numbers as described in Section 2. Retention was tested at 24 h. $**P<.01$.

vehicle. For comparison, animals received either CAP or vehicle in both hemispheres ($n=22–24$). Similarly, ANI (30 mM), a cytosolic protein synthesis inhibitor, was injected at 4 or 5 h post-training directly into either the left or right LPO, with the contralateral LPO receiving vehicle. For comparison, animals received either ANI or vehicle in both hemispheres ($n=22–26$) and recall was tested at 24 h. The concentrations of CAP and ANI used were based on our previous study [22].

2.3. Analysis of data

Chicks that avoided the bead on test were regarded as remembering the avoidance response, while those that pecked were regarded as showing amnesia. Results were expressed as the percentage of birds that exhibited recall (% avoidance) for the training task when tested at 24 h. Comparisons of retention between drug- and vehicle-injected birds were made using χ^2.

3. Results

None of the drugs tested produced any effect on the ability of chicks to discriminate between aversive (chrome bead) and non-aversive (white bead) stimuli, indicating no non-specific effects of these agents on normal neuronal functioning (results not shown).

3.1. Effect of FGIN 1–27 and PK 11195 on memory formation

The involvement of the MBR in long-term memory formation in the day-old chick was tested using the specific agonist, FGIN 1–27, and the specific antagonist, PK 11195, of this receptor. While FGIN 1–27 did not disrupt memory (Fig. 1A), PK 11195 caused amnesia when it was injected bilaterally into the LPO at concentrations of 1 and 10 μM ($P<.01$) and retention was tested at 24 h (Fig. 1B). Unilateral injections revealed that susceptibility to PK

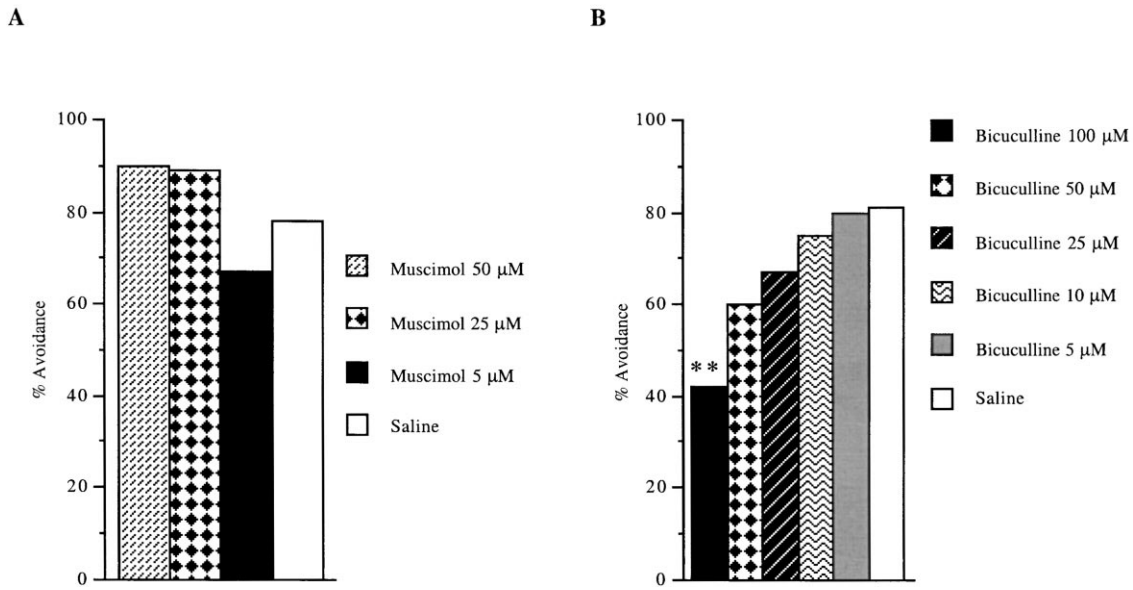

Fig. 3. Effect of bilateral intracranial injections, directly into the LPO (10 μl per hemisphere), 5 h after passive avoidance training of bicuculline or muscimol, $GABA_A$ receptor antagonist and agonist, respectively. Numbers as described in Section 2. Retention was tested at 24 h. $**P<.01$.

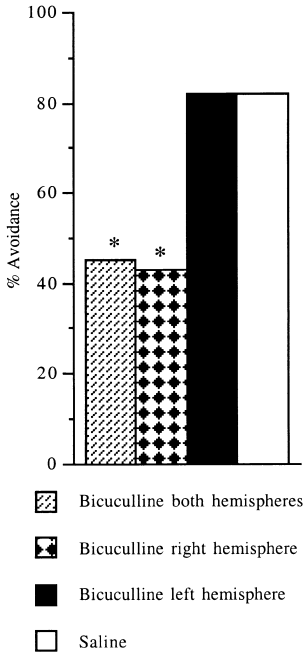

Fig. 4. Effect of unilateral intracranial injections directly into the LPO (10 μl per hemisphere) of 100 μM bicuculline at 5 h post-training. Numbers as described in Section 2. Retention was tested at 24 h. * $P<.05$.

11195 at this 5-h time point was confined to the right hemisphere, which includes the right LPO ($P<.01$) (Fig. 2). At the doses employed, neither PK 11195 nor FGIN 1–27 produced any detectable motor or behavioural abnormalities. To look for such abnormalities, we routinely observed the drug-injected chicks to verify that they had normal interest and ability to explore their environment by pecking, were not excessively sleepy, and did not emit distress calls.

3.2. Effect of muscimol and bicuculline on memory formation

Since the MBR is believed to regulate the $GABA_A$ receptor via its role in the control of steroid biosynthesis, we were interested to modulate the activity of the $GABA_A$ receptor directly and see if this had an effect on passive avoidance learning. Stimulation of the $GABA_A$ receptor by muscimol had no effect on retention of memory when injected bilaterally into the LPO at 5 h post-training. However, inhibition of the $GABA_A$ receptor by the injection of bicuculline at a concentration of 100 μM blocked memory formation ($P<.01$) (Fig. 3). Unilateral injections of bicuculline (100 μM) showed that the effect of bicuculline was confined to the right hemisphere ($P<.05$) (Fig. 4). In agreement with the results of Clements and Bourne [10], we found that 100 μM bicuculline or muscimol did not produce any behavioural abnormalities. These results indicate that the MBR could be affecting memory formation through its effects on the $GABA_A$ receptor.

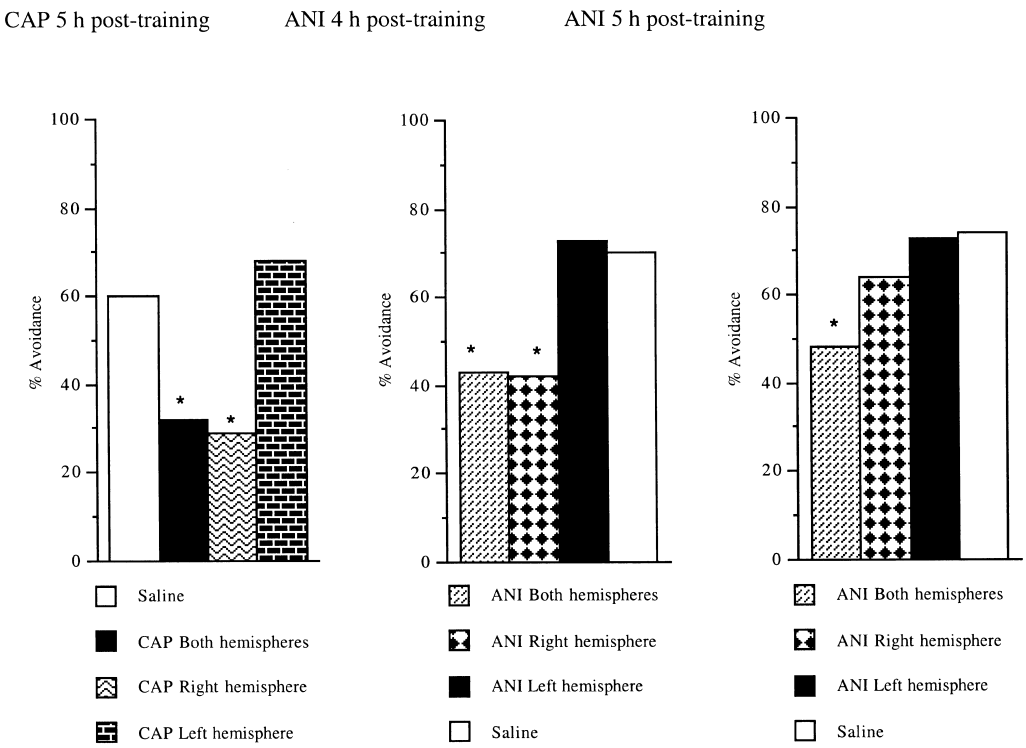

Fig. 5. Effect of unilateral intracranial injections directly into the LPO (10 μl per hemisphere) of (A) CAP at 5 h, or (B) ANI at 4 h or (C) ANI at 5 h post-training. Concentrations and numbers as described in text and Section 2. Retention was tested at 24 h. * $P<.05$.

3.3. Effect of unilateral injections of ANI and CAP on memory formation

As the antagonism of the MBR and the $GABA_A$ receptor indicated that the memory-forming processes appeared to be confined to the right LPO at 5 h after training, it was of interest to determine if such effects were also observed with two other inhibitors effective at this time point. These are the protein synthesis inhibitors, CAP and ANI, which block mitochondrial and cytosolic protein syntheses, respectively. CAP was effective in blocking memory at 5 h post-training when injected bilaterally ($P<.05$) or into the right LPO only ($P<.05$) (Fig. 5A). Similarly, both bilateral ($P<.05$) and right LPO injections ($P<.05$) of ANI disrupted memory at 4 h after training, but by 5 h, only bilateral injections caused amnesia (Fig. 5B and C).

4. Discussion

The results show that stimulation of the MBR by FGIN 1–27 does not disrupt memory formation for passive avoidance learning (Fig. 1A). However, when the receptor is inhibited by PK 11195, memory formation is affected (Fig. 1B). There is an inverse dose–response effect; high concentrations of the antagonist do not disrupt memory, but low concentrations do. There are two possible explanations for this effect. PK 11195 is known to also be a partial agonist [2,47] and at high concentrations, we may be seeing this agonistic property. Alternatively, the response may be a classical 'U'-shaped dose–response curve with the descending arm of the curve missing because we have not employed a concentration of PK 1195 that is low enough. 'U'-shaped dose–response curves for the effects of post-training drug injections on subsequent learning have been reported for many agents although the precise reasons for this property are as yet unclear [39,46]. Fig. 2 shows that the effect on memory due to PK 11195 is confined to the right hemisphere.

There have been a number of studies of the mechanisms that lead to MBR activation. It has been shown that FGIN 1–27 does not bind directly to other transmitter receptors, including $GABA_A$, $GABA_B$, glycine, glutamate, dopamine, serotonin, opiate, cholecystokinin, β-adrenergic, cannabinoid, or σ-receptors but is a specific agonist for the MBR [50,51]. FGIN 1–27 has been shown to antagonise the performance deficits induced by inhibition of the NMDA receptor in radial and water maze tests and in passive avoidance learning tasks. Thus, the NMDA receptor is implicated in the cascade of events that leads to MBR activation. Further experiments showed that the effect of FGIN 1–27 after passive avoidance training could be reversed by PK 11195 and the neurosteroid, pregnenolone sulphate [52]. Other studies have shown that morphine treatment or NMDA receptor activation is important in the regulation of DBI expression [31,41,52]. Little is known about which receptors are involved in the second wave of neuronal activity that occurs during passive avoidance learning in the chick. Thus, which receptors, if any, are involved with the regulation of the MBR via the DBI at 5 h in the chick remains to be determined.

Since upregulation of the MBR can indirectly affect the $GABA_A$ receptor via the synthesis of neurosteroids [50,51], we sought to investigate whether direct modulation of the $GABA_A$ receptor had an effect on passive avoidance learning at 5 h post-training. Stimulation of the $GABA_A$ receptor by muscimol had no effect on memory, but inhibition of the receptor with bicuculline disrupted memory formation (Fig. 3). Unilateral injections of bicuculline demonstrated that this effect is confined to the right hemisphere (Fig. 4). It, therefore, is possible that the involvement of the MBR in memory formation may be related, in part, to $GABA_A$ receptor modulation.

The density of GABA receptors is relatively high in the LPO [13,58–60]; the region of the brain where injections were made in the present work, and $GABA_A$ receptors have been shown to be upregulated in the LPO after learning [38]. GABAergic transmission is important for the retention of a number of different learning tasks in a variety of species [4,5,7,63]. Most studies show that the $GABA_A$ antagonist, bicuculline [14], enhances memory for avoidance tasks when injected around the time of training [3,4,6,30] and this is also true in the chick [10]. In the early stages of memory formation, there appears to be some evidence of an interaction of the GABAergic and the β-noradrenergic system in the rat and mouse and perhaps the chick [10,12,30].

Two other inhibitors which are effective at 5 h post-training have been described. These are CAP, an inhibitor of mitochondrial protein synthesis [22], and ANI, which blocks cytosolic protein synthesis. We were interested to see if the effect of these inhibitors on passive avoidance memory formation showed the same specificity of action in the right hemisphere and whether their action could plausibly be related to the MBR. The protein synthesis inhibitor, ANI, has been shown to disrupt memory when injected between 4 and 5 h post-training [21]. Fig. 5B shows that when ANI was injected at 4 h, this effect was confined to the right hemisphere, but an hour later, only bilateral injections disrupted memory formation (Fig. 5C), suggesting that either hemisphere was capable of "maintaining" the memory at 5 h post-training. A lack of effect with unilateral injections at 5 h is not due to ANI diffusion to the other hemisphere since there was lateralization to the right hemisphere when ANI was injected at 4 h. The inhibitor of mitochondrial protein synthesis, CAP, has been shown to disrupt memory when injected at 5 h after training only [22]. Here we show that this inhibition is confined to the right hemisphere (Fig. 5A).

While the major effect of CAP is to inhibit mitochondrial protein synthesis [48], it has also been shown to inhibit the activity of some members of cytochrome *P*450 family at

extremely high concentrations (300 mg/kg) [28]. It may be that CAP is not only inhibiting mitochondrial protein synthesis in the present studies. but is also affecting $P450_{ssc}$ activity. Loscertales et al. [35] have shown that the inhibitor of corticosterone, aminoglutethimide, disrupts passive avoidance memory when administered intraperitoneally prior to training and recall is tested at 24 h. This compound is also known to inhibit cytochrome *P*450 [42,62] including $P450_{ssc}$ [55]. Since this compound has been shown to have a long half-life in most species (approximately 24 h) [16,17,34], it is plausible that it is exerting its effect 5 h post-training. Sandi and Rose [54] showed that pre-training injections of corticosteroid receptor inhibitors only started to exert their effect from 4 h onwards. This raises the possibility that CAP could be inhibiting neurosteroid synthesis via inhibition of cytochrome $P450_{ssc}$ and thereby interfering with the role of the MBR. The effect of ANI could also relate to the MBR. Protein synthesis would be expected to be important for the production of endogenous peptide ligands that activate the MBR. Inhibitors of protein synthesis have been shown to block steroidogenic stimulation by hormones [49,56].

A more detailed understanding of the processes of memory formation occurring 5 h after training in the chick awaits further studies, but the present work has given clear evidence that these processes can be modulated by the MBR and $GABA_A$ receptor.

References

[1] Anholt RR, Pedersen PL, De Souza EB, Snyder SH. The peripheral-type benzodiazepine receptor. Localization to the mitochondrial outer membrane. J Biol Chem 1986;261:576–83.

[2] Benavides J, Guilloux F, Allam DE, Uzan A, Mizoule J, Renault C, Dubroeucq MC, Gueremy C, Le Fur G. Opposite effects of an agonist, Ro5–4864, and an antagonist, PK 11195, of the peripheral-type benzodiazepine binding sites on audiogenic seizures in DBA/2J mice. Life Sci 1984;34:2613–20.

[3] Brioni JD, McGaugh JL. Post-training administration of GABAergic antagonists enhances retention of aversively motivated tasks. Psychopharmacology (Berlin) 1988;96:505–10.

[4] Brioni JD, Nagahara AH, McGaugh JL. Involvement of the amygdala GABAergic system in the modulation of memory storage. Brain Res 1989;487:105–12.

[5] Castellano C, Brioni JD, McGaugh JL. GABAergic modulation of memory. In: Squire L, Lindenlaub E, editors. Biology of memory. New York: Schattauer Verlag, 1990. pp. 361–78.

[6] Castellano C, Brioni JD, Nagahara AH, McGaugh JL. Post-training systemic and intra-amygdala administration of the $GABA_B$ agonist baclofen impairs retention. Behav Neural Biol 1989;52:170–9.

[7] Castellano C, Introini-Collison IB, Pavone F, McGaugh JL. Effects of naloxone and naltrexone on memory consolidation in CD1 mice: involvement of GABAergic mechanisms. Pharmacol Biochem Behav 1989;32:563–7.

[8] Chen ZW, Bergman T, Jornvall H, Bonetto V, Norberg A, Mutt V, Longone P, Costa E, Efendic S, Ostenson CG. Full-length and N-terminally truncated chicken intestinal diazepam-binding inhibitor. Purification, structural characterization and influence on insulin release. Regul Pept 1997;69:63–8.

[9] Cherkin A. Kinetics of memory consolidation. Role of amnesic treatment parameters. Proc Natl Acad Sci USA 1969;63:1094–101.

[10] Clements MP, Bourne RC. Passive avoidance learning in the day-old chick is modulated by GABAergic agents. Pharmacol Biochem Behav 1996;53:629–34.

[11] Costa E, Guidotti A. Molecular mechanism in the receptor activation of benzodiazepines. Annu Rev Pharmacol Toxicol 1979;19:531–45.

[12] Crowe SF, Ng KT, Gibbs ME. Possible noradrenergic involvement in training stimulus intensity. Pharmacol Biochem Behav 1991;39:717–22.

[13] Csillag A, Stewart MG, Curtis EM. GABAergic structures in the chick telencephalon: GABA immunochemistry combined with light and electron microscope autoradiography, and Golgi impregnation. Brain Res 1987;437:283–97.

[14] Curtis DR. Bicuculline, GABA and central inhibition. Proc Aust Assoc Neurol 1973;9:145–53.

[15] Da Cunha C, Huang CH, Walz R, Dias M, Koya R, Bianchin M, Pereira ME, Izquierdo I, Medina JH. Memory facilitation by post-training intraperitoneal, intracerebroventricular and intra-amygdala injection of Ro5–4864. Brain Res 1991;544:133–6.

[16] Dalrymple PD, Nicholls PJ. Metabolism profiles and excretion of ^{14}C-aminoglutethimide in several animal species and man. Xenobiotica 1988;18:75–81.

[17] Dalrymple PD, Nicholls PJ. Tissue distribution and elimination of ^{14}C-aminoglutethimide in the mouse. Eur J Drug Metab Pharmacokinet 1990;15:31–5.

[18] Davis JL, Pico KM, Cherkin A. Memory enhancement induced in chicks by L-prolyl-L-leucine-glycineamide. Pharmacol Biochem Behav 1982;17:893–6.

[19] Edgar PF, Schonberger SJ, Dean B, Faull RL, Kydd R, Cooper GJ. A comparative proteome analysis of hippocampal tissue from schizophrenic and Alzheimer's disease individuals. Mol Psychiatry 1999;4:173–8.

[20] Freeman FM, Rose SPR. Expression of Fos and Jun proteins following passive avoidance training in the day-old chick. Learn Mem 1999;6:389–97.

[21] Freeman FM, Rose SPR, Scholey AB. Two time windows of anisomycin-induced amnesia for passive avoidance training in the day-old chick. Neurobiol Learn Mem 1995;63:291–5.

[22] Freeman FM, Young IG. Chloramphenicol-induced amnesia for passive avoidance training in the day-old chick. Neurobiol Learn Mem 1999;71:80–93.

[23] Gibbs ME, Ng KT. Psychobiology of memory: towards a model of memory formation. Behav Rev 1977;1:113–36.

[24] Gigg J, Patterson TA, Rose SPR. Training-induced increases in neuronal activity recorded from the forebrain of the day-old chick are time-dependent. Neuroscience 1993;56:771–6.

[25] Gigg J, Patterson TA, Rose SPR. Increases in neuronal bursting recorded from the chick lobus parolfactorius after training are both time-dependent and memory-specific. Eur J Neurosci 1994;6:313–9.

[26] Gilbert DB, Patterson TA, Rose SPR. Dissociation of brain sites necessary for registration and storage of memory for a one-trial passive avoidance task in the chick. Behav Neurosci 1991;105:553–61.

[27] Guidotti A, Forchetti CM, Corda MG, Konkel D, Bennett CD, Costa E. Isolation, characterization, and purification to homogeneity of an endogenous polypeptide with agonistic action on benzodiazepine receptors. Proc Natl Acad Sci USA 1983;80:3531–5.

[28] Halpert J, Balfour C, Miller NE, Morgan ET, Dunbar D, Kaminsky LS. Isozyme selectivity of the inhibition of rat liver cytochromes *P*450 by chloramphenicol in vivo. Mol Pharmacol 1985;28:290–6.

[29] Herzog CD, Stackman RW, Walsh TJ. Intraseptal flumazenil enhances, while diazepam-binding inhibitor impairs, performance in a working memory task. Neurobiol Learn Mem 1996;66:341–52.

[30] Introini-Collison IB, Castellano C, McGaugh JL. Interaction of GABAergic and beta-noradrenergic drugs in the regulation of memory storage. Behav Neural Biol 1994;61:150–5.

[31] Katsura M, Hara A, Higo A, Tarumi C, Hibino Y, Ohkuma S. Continuous treatment with morphine increases diazepam-binding inhibitor mRNA in mouse brain. J Neurochem 1998;71:2638–41.
[32] Korneyev A, Pan BS, Polo A, Romeo E, Guidotti A, Costa E. Stimulation of brain pregnenolone synthesis by mitochondrial diazepam-binding inhibitor receptor ligands in vivo. J Neurochem 1993;61: 1515–24.
[33] Krueger KE, Papadopdoulos V. Mitochondrial benzodiazepine receptors and the regulation of steroid biosynthesis. Annu Rev Pharmacol Toxicol 1992;32:211–37.
[34] Lindstrom TD, Whitaker GW. Disposition of the aromatase inhibitor, LY56110, and associated induction and inhibition studies in rats, dogs, and monkeys. Fundam Appl Toxicol 1987;8:595–604.
[35] Loscertales M, Rose SP, Sandi C. The corticosteroid synthesis inhibitors metyrapone and aminoglutethimide impair long-term memory for a passive avoidance task in day-old chicks. Brain Res 1997;769: 357–61.
[36] Lössner B, Rose SPR. Passive avoidance training increases fucokinase activity in right forebrain tissue of day-old chicks. J Neurochem 1983;41:1357–63.
[37] Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM. Steroid hormone metabolites are barbiturate like modulators of the GABA receptor. Science 1986;232:1004–7.
[38] Martijena ID, Arce A. Transient benzodiazepine $GABA_A$ receptor increase after passive avoidance learning in synaptosomal membranes from chick brains. Can J Physiol Pharmacol 1994;72:233–7.
[39] Martinez JJ, Schulteis G, Weinberger SB. How to increase and decrease the strength of memory traces: the effects of drugs and hormones. In: Martinez JL, Kesner RP, editors. Learning and memory: a biological view. New York: Academic Press, 1991.
[40] Mason RJ, Rose SPR. Lasting changes in spontaneous multi-unit activity in the chick brain following passive avoidance training. Neuroscience 1987;21:931–41.
[41] Mocchetti I, Costa E. In vivo studies of the regulation of neuropeptide stores in structures of the rat brain. Neuropharmacology 1987;26: 855–62.
[42] Nicholls PJ, Daly MJ, Smith HJ. Pharmacology of aminoglutethimide: structure/activity relationships and receptor interactions. Breast Cancer Res Treat 1986;7:S55–67 Supplement.
[43] Pallares M, Darnaudery M, Day J, Le Moal M, Mayo W. The neurosteroid pregnenolone sulfate infused into the nucleus basalis increases both acetylcholine release in the frontal cortex or amygdala and spatial memory. Neuroscience 1998;87:551–8.
[44] Patterson TA, Gilbert DB, Rose SPR. Pre- and post-training lesions of the intermediate medium hyperstriatum ventrale and passive avoidance learning in the chick. Exp Brain Res 1990;80:189–95.
[45] Patterson TA, Rose SPR. Memories in the chick: multiple cues, distinct brain locations. Behav Neurosci 1992;106:465–70.
[46] Patterson TA, Schulteis G, Alvarado MC, Martinez JL, Bennett EL, Rosenzweig MR, Hruby VJ. Influence of opioid peptides on learning and memory processes in the chick. Behav Neurosci 1989;103: 429–37.
[47] Pellows S, File SE. Behavioural actions of Ro5–4864: a peripheral-type benzodiazepine? Life Sci 1984;35:229–40.
[48] Pestka S. Inhibitors of ribosome function. Annu Rev Microbiol 1971;25:487–562.
[49] Privalle CT, Crivello JF, Jefcoate CR. Regulation of intramitochondrial cholesterol transfer to side-chain cleavage cytochrome *P*450 in rat adrenal gland. Proc Natl Acad Sci USA 1983;80:702–6.
[50] Romeo E, Auta J, Kozikowski AP, Ma D, Papadopoulos V, Puia G, Costa E, Guidotti A. 2-Aryl-3-indoleacetamides (FGIN 1): a new class of potent and specific ligands for the mitochondrial DBI receptor (MDR). J Pharmacol Exp Ther 1992;262:971–8.
[51] Romeo E, Cavallaro S, Korneyev A, Kozikowski AP, Ma D, Polo A, Costa E, Guidotti A. Stimulation of brain steroidogenesis by 2-aryl-indole-3-acetamide derivatives acting at the mitochondrial diazepam-binding inhibitor receptor complex. J Pharmacol Exp Ther 1993; 267:462–71.
[52] Romeo E, Cheney DL, Zivkovic I, Costa E, Guidotti A. Mitochondrial diazepam-binding inhibitor receptor complex agonists antagonize dizocilpine amnesia: putative role for allopregnanolone. J Pharmacol Exp Ther 1994;270:89–96.
[53] Rose SPR. How chicks make memories: the cellular cascade from c-*fos* to dendritic remodelling. TINS 1991;14:390–7.
[54] Sandi C, Rose SP. Corticosteroid receptor antagonists are amnesic for passive avoidance learning in day-old chicks. Eur J Neurosci 1994; 6:1292–7.
[55] Santen RJ, Misbin RI. Aminoglutethimide: review of pharmacology and clinical use. Pharmacotherapy 1981;1:95–120.
[56] Simpson ER, McCarthy JL, Peterson JA. Evidence that the cyclohexamide-sensitive site of adrenocorticotropic hormone action is in the mitochondrion. Changes in pregnenolone formation, cholesterol content, and the electron paramagnetic resonance spectra of cytochrome *P*450. J Biol Chem 1978;253:3135–9.
[57] Steele RJ, Stewart MG. Involvement of AMPA receptors in maintenance of memory for a passive avoidance task in day-old domestic chicks (*Gallus domesticus*). Eur J Neurosci 1995;7:1297–304.
[58] Stewart MG, Bourne RC. Ontogeny of [^{3}H] muscimol binding to membranes of the chick forebrain. Exp Brain Res 1987;65:707–10.
[59] Stewart MG, Bourne RC, Chmielowska J, Kalman M, Csillag A, Stanford D. Quantitative autoradiographic analysis of the of the distribution of [^{3}H] muscimol binding to GABA receptors in chick brain. Brain Res 1988;456:387–91.
[60] Veenman CL, Reiner A. The distribution of GABA-containing perikarya, fibers and terminals in the forebrain and midbrain of pigeons, with particular reference to the basal ganglia and its projection targets. J Comp Neurol 1994;339:209–50.
[61] Venault P, Chapoutier G, Prado de Carvalho L, Simiand J, Morre M, Dodd RH, Rossier J. Benzodiazepine impairs and β-carboline enhances performance in learning and memory tasks. Nature 1986;321: 864–6.
[62] Walther B, Ghersi-Egea JF, Minn A, Siest G. Brain mitochondrial cytochrome $P450_{scc}$: spectral and catalytic properties. Arch Biochem Biophys 1987;254:592–6.
[63] Yonkov DI, Georgiev VP. Memory effects of GABAergic antagonists in rats trained with two-way active avoidance tasks. Acta Physiol Pharmacol Bulg 1985;11:44–9.