Neuroscience 131 (2005) 567–576

OPPOSING ROLES FOR $GABA_A$ AND $GABA_C$ RECEPTORS IN SHORT-TERM MEMORY FORMATION IN YOUNG CHICKS

M. E. GIBBS[a]* AND G. A. R. JOHNSTON[b]

[a]*Department of Pharmacology, Monash University, Wellington Road, Clayton, Victoria 3800, Australia*

[b]*Department of Pharmacology, University of Sydney, Sydney, 2006, Australia*

Abstract—The inhibitory neurotransmitter GABA has both inhibitory and enhancing effects on short-term memory for a bead discrimination task in the young chick. Low doses of GABA (1–3 pmol/hemisphere) injected into the multimodal association area of the chick forebrain, inhibit strongly reinforced memory, whereas higher doses (30–100 pmol/hemisphere) enhance weakly reinforced memory. The effect of both high and low doses of GABA is clearly on short-term memory in terms of both the time of injection and in the time that the memory loss occurs. We argue on the basis of relative sensitivities to GABA and to selective GABA receptor antagonists that low doses of GABA act at $GABA_C$ receptors (EC_{50} approximately 1 μM) and the higher doses of GABA act via $GABA_A$ receptors (EC_{50} approximately 10 μM). The selective $GABA_A$ receptor antagonist bicuculline inhibited strongly reinforced memory in a dose and time dependent manner, whereas the selective $GABA_C$ receptor antagonists TPMPA and P4MPA enhanced weakly reinforced in a dose and time dependent manner. Confirmation that different levels of GABA affect different receptor subtypes was demonstrated by the shift in the GABA dose-response curves to the selective antagonists. It is clear that GABA is involved in the control of short-term memory formation and its action, enhancing or inhibiting, depends on the level of GABA released at the time of learning. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: GABA, $GABA_C$ receptor, $GABA_A$ receptor, short-term memory, chick, discriminated avoidance learning.

The neurotransmitter GABA is found primarily in inhibitory interneurons impinging on and modulating activity in the vertebrate brain. GABA is considered to play a controlling role on the balance of excitability and inhibitory states in the cortex and hippocampus and the interneurons involved are viewed as having an active role in information processing (Paulsen and Moser, 1998). GABA receptor agonists and antagonists have been variously reported to enhance or inhibit memory processing, but recently GABA has been implicated directly in cognitive processing (Leventhal et al., 2003) where a relationship between declining GABA levels and old age was demonstrated. An increase in visual function and discriminatory ability was found when GABA receptors were activated in the visual cortex in monkeys (Leventhal et al., 2003).

There are three major classes of GABA receptors in the CNS: $GABA_A$, $GABA_B$ and $GABA_C$. The pharmacology of $GABA_A$ and $GABA_B$ receptors has been extensively investigated (Johnston, 1996a), but $GABA_C$ receptors have been less well studied (Johnston, 1996b; Chebib and Johnston, 2000). $GABA_A$ and $GABA_C$ are both ionotropic receptors and activate chloride channels. $GABA_A$ receptors are inhibited by the alkaloid bicuculline whereas $GABA_C$ receptors are not (Johnston, 1996b). $GABA_B$ receptors are metabotropic, transmembrane receptors coupled to second messengers. They are activated by baclofen and blocked by phaclofen but not blocked by bicuculline. Relatively high levels of the $GABA_C$ receptor subunits ρ1 and ρ2 have been described in chick brain using *in situ* hybridization and RT-PCR (Albrecht et al., 1997). With the exception of δ-subunit containing $GABA_A$ receptors (Brown et al., 2002), GABA is an order of magnitude more potent on $GABA_C$ receptors than on $GABA_A$ receptors (Chebib and Johnston, 2000). The $GABA_C$ receptor antagonist TPMPA ((1,2,5,6-tetrahydropyridin-4-yl) methylphosphinic acid; Ragozzino et al., 1996) is some eight times more potent as an antagonist of human recombinant ρ1 than of ρ2 $GABA_C$ receptors (Chebib et al., 1998), while P4MPA ((piperidine-4-yl)methylphosphinic acid) shows similar potency as an antagonist of both ρ1 and ρ2 $GABA_C$ receptors (Johnston et al., 1998).

GABA is involved in many neurological and psychiatric disorders including the epilepsies and GABA uptake systems have recently become a therapeutic target for these disorders (Sarup et al., 2003). There have been a number of reports suggesting that removal of the influence of inhibitory GABA receptors (e.g. by bicuculline) leads to memory enhancement and conversely its activation by agents such as muscimol leads to memory inhibition. (Brioni and McGaugh, 1989; Brioni et al., 1989; Castellano et al., 1989; Izquierdo and Medina, 1991; Clements and Bourne, 1996) As all three GABA receptors are activated by the neurotransmitter, the question arises as to how is there selectivity in the action of GABA? We have recently demonstrated a role for $GABA_C$ receptors in cognitive processing using selective $GABA_C$ receptor antagonists (Johnston et al., 1998) which contrasts with the role of the selective $GABA_A$ receptor antagonist bicuculline on memory in the chick.

*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: DR, discrimination ratio; EC_{50}, concentration producing 50% maximal response; GABA, γ-aminobutyric acid; IMHV, intermediate medial hyperstriatum ventrale; IMM, intermediate medial mesopallium; ITM, intermediate memory; LTM, long-term memory; P4MPA, (piperidin-4-yl)methylphosphinic acid; STM, short-term memory; TPMPA, (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid.

0306-4522/05$30.00+0.00 © 2005 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2004.11.033

We have a model of memory formation in the chick based on behavioral and neuropharmacological experiments that outlines three sequential stages in memory formation derived from single trial passive and discriminated avoidance tasks (Gibbs and Ng, 1977; Gibbs and Summers, 2002). The task involves day-old chicks learning not to peck at a red bead but to continue pecking at a blue bead on test when the red bead was made to taste aversive on training (100% anthranilate). Establishment of memory follows a very reproducible time course: short-term memory (STM) lasting 10 min, is followed after a transient dip at 15 min, by intermediate memory (ITM) lasting from 20 to 50 min, and after a second transient dip at 55 min, by protein synthesis-dependent long-term memory (LTM). LTM formation is dependent upon consolidation of the memory trace at 30 min, a time point that corresponds to phase A at the beginning of ITM. The division of ITM into phase A and phase B has been established on pharmacological grounds (Gibbs and Ng, 1984). Consolidation does not occur if the aversive stimulus is weakened by reducing the anthranilate concentration from 100% to 20%. In this case memory is normal during the first 30 min after training but ITMB and subsequently LTM does not appear. In a series of recent papers (reviewed in Gibbs and Summers, 2002) we have established roles for five adrenoceptor subtypes in the modulation of memory by systematic investigation involving selective adrenoceptor agonists and antagonists and establishing the selectivity of the action of the agonists at the receptors. We have shown that adrenoceptors respond to the selective adrenoceptor agonists in different brain regions and that the receptors are inhibited by selective adrenoceptor antagonists at different times during the sequential stages of memory.

In this paper, we have used a similar approach to elucidate the action of the different GABA receptor subtypes. We examine the effect of central injection of GABA on memory and the action of selective $GABA_A$ and $GABA_C$ antagonists and their effects on exogenously administered GABA on memory in the chick. As with studies on the differential contributions $GABA_A$ and $GABA_C$ receptors to the actions of GABA in rat retinal slices by Euler and Wässle (1998), we used lower doses of GABA to activate $GABA_C$ receptors and higher doses to activate $GABA_A$ receptors, together with selective $GABA_A$ and $GABA_C$ receptor antagonists.

The procedures outlined in this paper are approved by the Monash University Animal Ethics Committee and comply with the 1997 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. All efforts were made to minimise both the suffering and the number of animals used.

EXPERIMENTAL PROCEDURES

Animals

Up to 240 1- to 2-day old Black Australorp×White Leghorn male chickens were delivered from a local poultry farm (Research Poultry Pty. Ltd, Research, Victoria, Australia) on the morning of each experiment. Full details of the experimental protocol are to be found in Gibbs and Summers (2002). The chicks were placed in pairs, given *ad libitum* access to chick crumbs scattered on the floor of their box. Between 16 and 20 chicks were allocated to each experimental group. At the end of each experiment animals may be excluded on the basis of not training or not pecking at the control bead on test.

Drugs and injections

GABA, the selective $GABA_C$ antagonists TPMPA and P4MPA (prepared as described by Hanrahan et al., 2001) and the selective $GABA_A$ antagonist (−)-bicuculline (Sigma) were made up in sterile physiological saline. Intracranial injections were administered by freehand injection of 5 or 10 μl per hemisphere, aimed at the intermediate medial hyperstriatum ventrale (IMHV) or mesopallium (IMM) of the forebrain at a depth of 3.5–4 mm using a Hamilton Repeating Dispenser syringe, with a stop on the 27gauge needle to control the depth of injection. This area of the chick forebrain has been shown to be metabolically active following imprinting (McCabe and Horn, 1994) and after passive avoidance training (Rose and Csillag, 1985; Sedman et al., 1992). Dose-response curves were constructed to bicuculline and P4MPA for s.c. administration for specificity challenges. In these cases, the drugs were injected in 100 μl volumes into a fold of skin on the ventral surface of the chick. In this paper we will use the new nomenclature introduced by Reiner et al. (2004) to describe the brain regions in the bird.

Learning paradigm

The procedures have been described in detail elsewhere (Gibbs and Ng, 1977; Gibbs and Summers, 2002). Briefly, chicks were exposed to novel objects coming into their cage, to start with small chrome 2 mm diameter beads, and then larger red- and blue-colored glass beads which had been dipped in water. This was to encourage the chicks to peck freely at beads presented to them and to avoid fear of strange objects coming into their cage. This procedure reduces the variability in subsequent tests and ensures that all chicks will peck at both colored beads. Training consisted of presentation of a red bead after dipping it in the chemical aversant, methyl anthranilate. After tasting anthranilate chicks typically shake their heads and wipe their beaks on the floor. Once they have registered the taste they will not peck at it again in the 10 s trial. On retention testing, the chicks are presented with a clean red bead, followed by a clean blue bead 2.5 min later, and allowed 10 s to peck at each bead. The number of pecks at the beads and the latency to the first peck, in all trials with the colored beads, are recorded by on-line computer. The drugs were administered at predetermined times relative to the learning trial and testing was also relative to the learning trial.

At the completion of the experiment, data were retrieved from the computer and results calculated. Chicks failing to peck at the red training bead or failing to peck at the blue bead on test were excluded from further analysis. Exposing chicks to the presentation of beads into their cage before the training trial reduces problems of generalized non-pecking of beads. The exclusion of chicks on the basis of failing to peck the training bead or the blue test bead is made at the conclusion of the experiment.

Statistics

Memory was indexed by a discrimination ratio (DR) defined as the ratio of the number of pecks at the blue bead to the total number of pecks at the red and the blue bead, for any chick which pecked at the blue bead on the retention test (Ng and Gibbs, 1991). A DR approaching 1.0 indicates good memory and a tendency to avoid or reduce pecking at the red bead, whereas a DR approaching 0.5 indicates equal pecking at both red and blue beads. The number of pecks on the blue bead can reach up to 10 or more in the 10 s trial.

All statistical tests were carried out with type 1 error rate set at $\alpha=0.05$, and statistical values are reported to three decimal