Neuroscience 230 (2013) 62–71

DELETERIOUS EFFECTS OF SOLUBLE BETA AMYLOID ON COGNITION, ANTAGONISM BY SALINE AND NORADRENALINE, A ROLE FOR MICROGLIA

M. E. GIBBS [a,b]* **AND C. L. GIBBS** [c]

[a] *Monash Institute of Pharmaceutical Sciences, 399 Royal Parade, Parkville, Victoria 3052, Australia*

[b] *Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria 3800, Australia*

[c] *Emeritus Professor, Department of Physiology, Monash University, Clayton, Victoria 3800, Australia*

Abstract—Small oligomeric beta amyloid ($A\beta_{1-42}$) injected 45 min prior to single-trial bead discrimination training resulted in impaired learning in day-old chickens. A new experimental protocol was used where the injections of drugs were at times around the time of injection of Aβ. It was found that the Na^+ levels of the saline used to dissolve Aβ affected cognitive impairment. Na^+ levels above the normal plasma value (140 mM) reduced Aβ-induced learning deficits whereas levels below increased sensitivity to Aβ. The new protocol was also used to examine the ability of certain noradrenergic adrenoceptor antagonist and agonists, insulin, glucose and minocycline to reduce learning disruption caused by Aβ. The drugs (made up in 154 mM sodium chloride) were injected *before*, at *the same time* or *after* the injection of Aβ and although all drugs prevented Aβ-induced disruption of learning when given in the same injection as Aβ, some injected before could prevent Aβ disrupting learning, whereas others could rescue learning ability when given after Aβ injection. These results are interpreted in the light of possible actions of noradrenaline on microglia and various processes: astrocytic metabolism, cerebral microcirculation, and removal of Aβ away from the site of injection. The possible importance of hypernatremia and hyponatremia in the incidence of Alzheimer's disease is discussed.

Key words: physiological saline, hyponatremia, chick, adrenoceptors, noradrenaline, microglia.

INTRODUCTION

Small soluble oligomeric beta amyloid (Aβ), rather than amyloid plaques, play a principal role in the cognitive deficits of Alzheimer's disease (AD) and may be the overriding cause in the early prodromal stages of AD which are characterized only by the impairment of the consolidation of new memories (Mileusnic et al., 2007; Walsh and Selkoe, 2007). We, and others, have documented the effects of soluble Aβ on learning ability using the young domestic chick as a model (Mileusnic et al., 2007; Gibbs et al., 2009, 2010b). $A\beta_{1-42}$ injected as early as 5 min prior to training resulted in memory loss due to failure to consolidate memory 35 min later, and maybe even earlier (Gibbs et al., 2010b). We found that Aβ-induced memory loss could be prevented by the noradrenergic β_3-adrenoceptor (-AR) agonist CL316243 suggesting that astrocytes may be involved in the rescue. Activation of this AR increases glucose uptake in astrocytes but not in neurons (Gibbs et al., 2008a; Hutchinson and Gibbs, unpublished). Further experiments showed that Aβ impairment of memory could be rescued by agents known to facilitate astrocytic oxidative metabolism (Gibbs et al., 2010b). In most of these experiments Aβ was injected 45 min before training (old protocol, Fig. 1).

In the present experiments we have used a new experimental protocol (new protocol, Fig. 1), to see if the effect of Aβ could be prevented by administering various drugs, including AR agonists and an AR antagonist, insulin and also glucose close to the time of Aβ injection. In the old protocol, Aβ was given 45 min before training but the drugs were given *after* training (Fig. 1) (Gibbs et al., 2009, 2010b). In the current experiments we examine the effect of drugs injected: (a) at the same time as Aβ, (b) just before Aβ to see if the effects of Aβ could be prevented, or (c) given just after Aβ to see if the effect of Aβ could be ameliorated.

We normally dilute the Aβ in physiological saline (154 mM sodium chloride (NaCl)), but we inadvertently used a higher dose of NaCl as an 'Aβ saline control' and in these experiments we were surprised to find that the slightly higher NaCl levels seemed to prevent the effects of Aβ. These serendipitous results led us to examine the effects of a range of different sodium levels on the action of Aβ on learning. Rather to our surprise, using this new experimental protocol with soluble Aβ made up in different NaCl molarities we found that small changes of sodium could alter the efficacy of Aβ. We established many years ago that quite small changes in various extracellular cations can alter memory processing, with memory being particularly sensitive to small changes in extracellular potassium (Gibbs et al.,

*Correspondence to: M. E. Gibbs, Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria 3800, Australia. Tel: +61-3-59684163.
E-mail addresses: marie.gibbs@monash.edu, marie.gibbs@med.monash.edu.au (M. E. Gibbs).

Abbreviations: Aβ, beta amyloid; AD, Alzheimer's disease; AR, adrenoceptor; DMSO, dimethyl sulfoxide; DR, discrimination ratio; IMM, intermediate medial mesopallium; NaCl, sodium chloride; OM, oxymetazoline.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2012.10.070

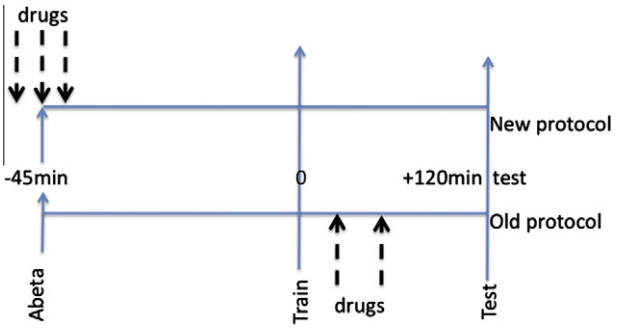

Fig. 1. Schematic of the two protocols for testing the effects of Aβ on learning. In both protocols Aβ is injected into the intermediate medial mesopallium, the avian equivalent of the mammalian cortex and an area involved intimately in memory processing 45 min before training (time 0) and the chicks are tested 120 min after training. The difference is that in the new protocol (top portion) agents are injected before, simultaneously with or just after the Aβ to prevent or ameliorate the effects of Aβ on learning. In the older Aβ protocol (bottom) the agents are injected after training to rescue memory.

1978), but in these same experiments we also noted that 140 mM Na^+ was optimal for memory consolidation. Perhaps this finding is not too surprising as it is the physiological Na^+ level found in CSF and plasma in chickens and in man (Anderson and Hazelwood, 1969). In fact 154 mM 'physiological' saline is actually hypernatremic. In humans the normal level of Na^+ is 142 (136–146 mM) and both hyponatremia (Na^+ levels < 135 mM) and hypernatremia (Na^+ levels > 145 mM) are known to affect brain function.

Our data suggest that as the extracellular Na^+ level is increased from 140 to 160 mM, there is a decrease in the ability of Aβ to impair memory, although this memory protecting activity can be overcome by raising the concentration of Aβ. We have shown that there is a correlation between the concentration of physiological levels of Na^+ and memory in the simultaneous presence of Aβ. This result, i.e. that higher levels of Na^+ within the mild hypernatremia range, are protective against the actions of Aβ is in accord with suggestions that hyponatremia increases the risk of AD (Marlow, 2009). To test the hyponatremic suggestion, we injected different concentrations of Aβ made up in 120-mM NaCl and found that the effects of Aβ were potentiated.

We stress again that in these experiments a new protocol was being used where drugs are given close to the time of administration of Aβ rather than to the time of learning and we have re-tested agents that we have used previously (old protocol) to consolidate memory and alleviate the effect of Aβ.

Noradrenergic dysfunction is implicated as an early symptom of AD and there are suggestions that AD originates in the locus coeruleus and that dysfunction of the noradrenergic input from the locus coeruleus to the cortex and hippocampus plays an important role in the cognitive impairment seen early in AD (e.g. Hertz, 1989; Marien et al., 2004; Weinshenker, 2008; Sanchez et al., 2011). As the locus coeruleus is the site of most of the noradrenergic neurons in the brain and sends projections to all parts of the brain including the hippocampus and cortex, it is clearly important in the noradrenergic modulation of memory. In the chick, activation of certain AR subtypes (β_2- and β_3-ARs) in the hippocampus and the cortical region following inhibition of processing in the locus coeruleus can rescue memory (Gibbs et al., 2010a). Others have also shown that increasing noradrenergic signalling can restore cognitive function in transgenic mouse models of AD (Sanchez et al., 2011).

In memory processing noradrenaline impacts on astrocytic as well as neuronal function (Gibbs et al., 2008a,b). Astrocytes have been implicated in the progression of AD (Iram and Frenkel, 2012) mediating the clearance of Aβ via transport through the blood–brain barrier, as well as secreting Aβ degrading enzymes (Kong et al., 2010; Iram and Frenkel, 2012). It has been suggested that Aβ causes inflammatory responses that prevent the microglial clearance of the peptide and could lead to retention of Aβ within the brain and noradrenergic receptors on microglia may be implicated. Therefore, in the present experiments using the new protocol, we examined whether any of the AR subtype agonists and antagonists that affect memory have any effect on the ability of Aβ to impair learning.

As soluble Aβ is present in the interstitial fluid of the AD brain, coming from leakage from Aβ deposits (plaques and tangles) rather than being newly synthesized (Kong et al., 2010), it remains plausible that pharmacological intervention even after the first signs of AD appear could have effect.

EXPERIMENTAL PROCEDURES

Materials

$A\beta_{1-42}$ (> 95% purity) was purchased from Rpeptides Inc. (Bogart, GA, USA). The peptide was made up as a 2-mM stock solution in dimethyl sulfoxide (DMSO) and then stored frozen at −20°C in 10 l aliquots. Prior to use, the Aβ was diluted with DMSO to 200 M. Thirty minutes prior to injections, Aβ was diluted either with physiological saline (0.9%, w/v, NaCl) for Aβ injection or for simultaneous administration in drugs made up in saline to yield the appropriate concentration according to each respective experiment. NaCl was also used in concentrations of 140 and 120 mM made by diluting physiological saline with distiled water. In other cases the drugs were made up in saline. Controls were injected with the appropriate dilution of DMSO. Zinterol was a gift from Bristol-Myers Squibb (Noble Park, Vic., Australia). Oxymetazoline (OM), insulin, prazosin, minocycline hydrochloride, and CL316243 (disodium (R,R)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate) were purchased commercially (Sigma–Aldrich Inc., St Louis, MO, USA). NaCl was obtained from Merck Australia (Australia). Physiological saline as 0.9% NaCl for intravenous infusion came from Baxter Healthcare (Australia).

Injections of peptide and drugs

Drugs and Aβ were given centrally by direct injection of 5 µl into the avian 'cortical' region (intermediate medial mesopallium, IMM) of each hemisphere using a 250 µl repeating Hamilton syringe dispenser. Unless otherwise indicated, each injection contained 10 pmol of Aβ peptide. The injections were performed freehand using the tactile landmarks of bregma and midline to target the injection site, i.e. 3 mm from the midline and 4–5 mm forward of bregma. The depth of the injection was