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Role of α7 Nicotinic Acetylcholine Receptors in the Pressor Response to Intracerebroventricular Injection of Choline: Blockade by Amyloid Peptide Aβ1-42

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Abbreviations:

amyloid beta peptide (1-42)	Aβ1-42
dihydro-β-erythroidin	DHβE
intracerebroventricular	icv
methyllycaconitine	MLA
nicotinic acetylcholine receptor	α7nAChR

Abstract

Systemic blood pressure and cardiac function have long been known to be under the control of central autonomic and hormonal pathways that, in part, utilize cholinergic neural systems. Recently choline, a precursor and product of acetylcholine metabolism, has been shown to serve as a selective endogenous agonist for the α7 subtype of the nicotinic acetylcholine receptor (α7nAChR). This receptor subtype mediates several responses to nicotine in animals, most notably, neuroprotection and enhanced cognition. The purpose of this study was to determine whether the cardiovascular changes induced by central injection of choline in rats also were mediated by α7nAChRs. Moreover, we sought to determine whether these cardiovascular changes to choline could be blocked by central pretreatment with Aβ1-42, a neurotoxic component of cerebral amyloid that is known to bind with high affinity to α7nAChRs. Central, intracerebroventricular (icv) injection of choline (50, 100 or 150 μg) produced dose-dependent (10-15 min duration) pressor response of up to about 20 mmHg. The most consistent change in heart rate included a brief increase (up to 40 beats/min) that lasted 2-3 min, followed by a prolonged decrease averaging 50 beats/min that lasted up to 30 min. Pretreatment (icv) with the selective α7nAChR antagonists α-bungarotoxin and methyllycaconitine (MLA) significantly inhibited the pressor and heart rate responses to subsequent injection of choline. Pretreatment with the non-α7-preferring antagonist dihydro-β-erythroidin (DHβE) was not effective. These findings suggested that the cardiovascular response to icv injection of choline was mediated at least in part through α7nAChRs. Pretreatment (30 min) with low doses (1 – 100 pmoles) of amyloid peptide Aβ1-42 (but not with Aβ40-1) administered by the icv route significantly inhibited the choline-induced blood pressure increase as well as the choline-induced decrease in heart rate.

One of the most prominent pathological changes associated with the Alzheimer's disease (AD) is the loss of cholinergic innervation of the cortex and hippocampus. This neuronal loss is accompanied by a decrease in the brain density of the α7 subtype of the nicotinic acetylcholine receptor (α7nAChR). The α7nAChR, is a unique member of the nicotinic receptor family which in recent years has been shown to enjoy significant physiological importance. In particular, this receptor subtype is known to mediate several responses to nicotine administration in animals, including neuroprotection and cognitive enhancement. For example, α7nAChR preferring agonists, such as anabaseine and GTS-21, were shown to improve working memory in rats (Meyer et al., 1994; Arendash et al., 1995; Briggs et al., 1997). Another α7nAChR agonist, ARR 17779, was shown to reverse the working memory deficits induced by fimbria-fornix lesions (Levin et al., 1999). However, other than procedures that estimate aspects of behavior, no objective measure of central α7nAChR activation *in vivo* has been described.

The peripheral circulation and cardiac function, to varying degrees and under specific conditions, are continuously regulated through the output of central autonomic and hormonal pathways. Although many important questions remain unanswered, a great level of understanding has been achieved concerning the central control of blood pressure. In this complicated and interconnected regulatory system, central cholinergic neural pathways have been shown to play important roles; and in unanesthetized rats, cholinergic agonists, both muscarinic (see, Brezenoff, 1982; Buccafusco, 1992) and nicotinic (Brezenoff and Giuliano, 1982; Buccafusco and Yang, 1993; Khan et al., 1994), have been shown to induce increases in arterial blood pressure. Changes in heart rate often are unpredictable, depending upon the particular agonist, and upon ongoing arousal and activity (Magri and Buccafusco, 1988). Some years ago, Caputi

and Brezenoff (1980) demonstrated that central intracerebroventricular (icv) injection of choline induced a short lasting, but significant increase in arterial pressure and a much longer lasting bradycardic response in unanesthetized rats. They failed to inhibit the pressor response through pretreatment of the animals by icv injection with either the muscarinic antagonist atropine or the nicotinic antagonist mecamylamine. Neither drug alone nor their combination modified the pressor response. However, neither of these compounds is subtype selective within their respective class, and atropine induces acetylcholine release. In light of the recently discovered ability of choline to serve as a selective agonist at α7nAChRs (Papke et al., 1996; Alkondon et al., 1999; Pereira et al., 2002), it is reasonable to consider that this receptor subtype mediates the choline-induced pressor response. With reasonably subtype selective nAChR antagonists now available this possibility can readily be examined. Thus, the first portion of this study was directed at determining whether the cardiovascular response to icv administration of choline was mediated predominantly through activation of α7nAChRs.

In the Alzheimer's disesase (AD) brain large deposits of amyloid plaques are present that consist chiefly of amyloid β peptides. In fact, recently, amyloid β peptides containing a crucial binding sequence, including the neurotoxic Aβ1-42, have been shown to bind with high affinity to α7 nAChRs (Wang et al., 1999; Wang et al., 2000). Moreover, Aβ and α7nAChRs appear to co-localize within the AD brain. Aβ peptides have been shown to inhibit the cellular responses to nAChR agonists (Pettit, et al., 2000; Liu, et al., 2001) and to prevent the cytoprotective action of nicotine *in vitro* (Li and Buccafusco, 2003). There also exists evidence to suggest that amyloid deposition in AD-related transgenic mouse strains alters the expression of nicotinic receptors *in vivo* (Bednar, et al., 2002). Whereas Aβ peptides have been directly introduced into the

cerebrospinal fluid of rodents by prolonged icv administration to elevate brain amyloid and to produce neurotoxicity, regimens specific for pharmacological interactions between the peptide and α7nAChRs *in vivo* have not been determined. Therefore, the second portion of this study was designed to assess the ability of Aβ peptides to inhibit the magnitude and expression of the acute pressor response to choline. In addition to the practical application of the study, these results may provide further *in vivo* evidence for the ability of Aβ peptides to target α7nAChRs, a subtype which is highly expressed on neural cells known to be selectively vulnerable to the toxic consequences of AD (Davies and Feisullin, 1981; Sugaya, et al., 1990; Hellstrom-Lindahl, et al., 1999).

Material and Methods

Materials

Choline iodide, methyllycaconitine citrate (MLA), atropine sulfate, amyloid β peptide 1-42, amyloid β peptide 40-1, and α-bungarotoxin were obtained from Sigma Chemicals, St. Louis, MO. Dihydro-β-erythriodine (DHβE) was donated by Merck & Co., Inc, Rahway, NJ. The doses used in this study for each nicotinic receptor antagonist were derived from previous studies in which they were shown to exert nicotinic receptor blocking actions in vivo with relative selectivity (Buccafusco and Yang, 1993; Jonnala et al., 2002). The β amyloid peptides were dissolved in sterile distilled water at 100 μM, and they were stored as aliquots at -20°C. They were incubated at 37°C for 24 hr before use to allow for aggregation.

Experimental Animals:

Male Wistar rats (Harlan Sprague-Dawley, Indianapolis, IN), weighing 280 – 380 g were housed in an environmentally controlled room on a 12-hr/12-hr day/night cycle and they were maintained on standard rat chow and tap water (unlimited). All animal protocols were previously approved by the Medical College of Georgia Committee on Animal Use for Research and Education.

Surgical Procedures:

Rats were anesthetized initially with methohexital (sodium), 65 mg/kg, i.p. and supplemented as needed. The subject's head was positioned in a stereotaxic frame with the skull in a horizontal position. A midline incision was made to expose the skull. Using stereotaxic landmarks a burr hole was placed over the left lateral cerebral ventricle. An additional burr hole was placed to the

right of the first hole and a stainless steel anchoring screw was inserted. A sterile stainless steel or cannula guide was attached to a micromanipulator on the stereotaxic frame. Each guide (Plastics One, Roanoke, VA) had a stainless steel shaft equivalent to 22 gauge tubing which was inserted into a wider threaded region made of Teflon at the top of the guide. The threaded region permits the introduction of a screw cap and dummy cannula to plug the guide when not in use. The cannula guide (11 mm long) was lowered to a depth of 4.0 mm below the skull through the burr hole over the ventricle. The tip of the guide was placed over, but without penetrating, the ventricular space. While in position, the guide and screw were covered with acrylic cement. After complete hardening of the cement, the manipulator was retracted and the guide plugged and capped. The skin was pulled around (but not over) the guide and sutured closed.

One week later rats again were anesthetized and a surgical incision was made in the midline of the abdomen to expose the lower portion of the abdominal aorta. A small nick was made in the lower portion of the aorta and a telemetry catheter (implant model TA11PA-C40, Data Sciences International, St. Paul, MN) was introduced and advanced until the tip rested below the origin of the renal arteries. The tip of the catheter contains a non-thrombogenic gel to prevent clot formation. The catheter end was fixed in place in the aorta by using Vetbond tissue adhesive (3M Animal Care Products, St. Paul, MN). The transmitter end of the catheter was placed subcutaneously over the abdomen and the wound was sutured closed.

Measurement of mean arterial pressure and heart rate:

Blood pressure and heart rate were measured using the DSI PhysioTel ® telemetry system (Data Sciences International, St. Paul, MN), which monitored the animals while they moved freely

within their cages. Each implanted transducer/transceiver transmitted pre-calibrated digitized blood pressure data via radio frequency signals to a nearby receiver. The data was sampled continuously, and by using the Dataquest ® data acquisition system (Data Sciences International, St. Paul, MN) which also calculated heart rate continuously from the pressure waveforms. Mean arterial pressure (MAP) and heart rate values were averaged and presented graphically in 30 sec bins.

Microinjections:

For icv injections, a 50 µl Hamilton micro-syringe (Reno, NV) was connected to a 28 gauge internal cannula (Plastics One, Inc., Roanoke, VA), which was inserted into the cranial 22 gauge cannula guide so that the tip rested within the lateral ventricle. All test compounds were dissolved in 10 µl sterile normal saline (vehicle), and they were delivered by hand over a period of 15-20 sec. At the completion of experiments each injection site was verified by injecting 10 µl India ink. The pretreatment drugs (or vehicle) were administrated 10 or 30 min (as indicated) prior to the choline (or vehicle) injection. Rats were allowed to recover for at least one day after each experiment (i.e., one choline microinjection). Each rat was used in no more than 5 experiments.

Statistical analysis:

The data depicted in the text and figures is presented as mean ± S.E.M. The existence of a significant difference between or among experimental groups was determined by analysis of variance with repeated measures. A modified *t* test with Bonferroni's correction for multiple comparisons using the error mean-square term from the analysis of variance was used for

analysis between groups of data. The criterion for statistical significance was $P<0.05$ for all comparisons.

Results

Because all of the data derived from this study are presented as the absolute change from resting (baseline) MAP and heart rate, Table 1 provides the baseline means for each of the experimental paradigms. There were no significant differences among the means for MAP [($F(10,65)=1.25$, $P=0.28$]. There also were no significant differences among means for resting heart rate [($F(10,65)=0.50$, $P=0.89$]. Thus, any change noted below in the magnitude or duration of the choline induced cardiovascular responses produced by pretreatment drugs could not be attributed to alterations in baseline (pre-choline) MAP or heart rate.

Microinjection of 10 μl of vehicle (sterile saline) on average produced only a brief (<3 min) increase in mean arterial pressure (MAP) of up to 3 mmHg. Microinjection of solutions of choline (50, 100, or 150 μg) within the same volume produced immediate and dose-dependent increases in MAP ranging from 10 to 20 mmHg (Fig. 1). There was a significant between dose component of the ANOVA [$F(2,10)=5.58$, $P=0.024$]. The responses peaked 3-4 min after injection and returned to pre-injection levels within 10-15 min. In some animals, MAP declined below preinjection values towards the end of the observation period. Simultaneously, heart rate initially increased (to about 40 beats/min) and it peaked about the same time as the increase in MAP (Fig. 2). Thereafter, heart rate declined to below pre-injection values. The late decrease in heart rate may reflect a decrease in cardiac output driving the late fall in MAP observed in some animals. Although the heart rate changes were not statistically dose-dependent [$F(2,10)=1.67$, $P=0.24$], the profile of response to choline was quite similar to that reported 13 years ago by Caputi and Brezenoff (1980) except for the brief initial increase in heart rate which they did not report observing. It is possible that the increase in heart rate may have reflected

animal activity just after the injection as it was not present in all animals. As observed by Caputi and Brezenoff (1980), and in this study, the duration of the decrease in heart rate usually far exceeded (at least up to 30 min) the duration of the increase in MAP. Also, soon after choline administration, an increase in locomotor activity was often observed, with the effect quickly diminishing; and the rats actually remained quiet, possibly sedate for the remainder of the observation period. Although the behavioral effects were not quantified, they were not apparent after icv injection of vehicle.

To determine whether α7nAChRs contribute to the cardiovascular response produced by choline, rats were pretreated by icv injection either with methyllycaconitine (MLA) or α-bungarotoxin, both α7nAChR-specific antagonists. In the first series, administration of 10 μg MLA (10 min prior to 150 μg of choline) resulted in a complete abolition of the pressor response to choline. Likewise, the biphasic heart rate change after choline was essentially normalized by MLA pretreatment (Fig. 3). In contrast, pretreatment with 0.3 μg atropine failed to significantly modify the pressor response to choline. This icv dose of atropine was shown previously to completely block the pressor response to central microinjection of muscarinic agonists (Caputi and Brezenoff, 1980). Atropine pretreatment partly, but significantly, reversed the changes in heart rate produced by choline. The administration of 10 μg α-bungarotoxin (30 min prior to 150 μg of choline), like MLA, significantly inhibited the magnitude of the choline-induced pressor response. These data are presented in Figure 4. The longer pretreatment time for α-bungarotoxin in this series was in consideration of the drug's slow binding kinetics. Toxin pretreatment significantly inhibited the choline-induced initial increase in heart rate, but it had no effect on the subsequent bradycardic response (Fig 4, inset). To further examine the specificity

of the choline response, the β2-subtype preferring antagonist DHβE was used as the pretreatment agent. Unlike MLA and α-bungarotoxin, pretreatment with 6 μg of DHβE failed to significantly inhibit the pressor response to subsequent injection of 150 μg of choline (Fig. 4). The heart rate changes induced by choline also were not affected by DHβE (Fig. 4, inset).

With the data suggesting that the initial pressor response to icv microinjection of choline was mediated predominantly by α7nAChRs, we next sought to determine whether pretreatment with the amyloid peptide (also known to block α7nAChRs) would effectively inhibit the choline response. Aβ1-42 (1, 10, or 100 pmol) was administrated 30 min prior to choline. The peptide itself elicited no change in resting MAP or heart rate (Table 1). The two higher doses of Aβ1-42 (10 pmol and 100 pmol) significantly inhibited the increases in MAP produced by icv injection of 150 μg of choline (Fig. 5). Under the same conditions the reverse peptide Aβ40-1 (100 pmol) failed to significantly affect the pressor response to choline (Fig. 6, upper panel). As with the receptor antagonists, the higher dose of the Aβ1-42 (100 pmol) normalized the biphasic heart rate response to icv injection of choline, while the same dose of the reverse peptide did not (Fig 6, lower panel).

Discussion

α7nAChRs have been suggested to participate in important higher brain functions such as working memory (Addy et al., 2003), in cell survival and neuroprotection (Li et al., 2000; Shimohama et al., 2001; Jonnala and Buccafusco, 2001), and to serve as a potential drug target in disease states such as schizophrenia (Marutle et al., 2001; Araki et al., 2002), epilepsy (Gil et al., 2002), Alzheimer's disease (Meyer et al., 1997; Briggs et al., 1997; Kem, 2000), and Parkinsons's disease (Burghaus et al., 2003). As new α7nAChR compounds become available it would be advantageous to have an efficient, objective, and experimentally simple model for drug efficacy. In this study, the most technically challenging procedure was the installation of the aortic blood pressure telemetry catheter. However, similar results may be obtained by using a standard catheter inserted into any major artery. In fact, for our very first dose finding experiments we directly catheterized the iliac artery and exteriorized the catheter to a water-tight swivel for the measurement of MAP in unanesthetized animals as we have described previously (Buccafusco and Yang, 1993). The use of the telemetry catheter was somewhat more advantageous as there are no exterior catheters to maintain, and the preparation has a longer useful lifetime.

The most reproducible response produced by icv microinjection of choline was the pressor response that occurred during the first 10-15 min after injection. Except for the magnitude of the pressor response (which was greater than that to choline), the cardiovascular changes to icv administered nicotine as we described them several years ago (Buccafusco and Yang, 1993) were quite similar to those produced by icv administration of choline. The maximal pressor response to nicotine (averaging about 30 mmHg) was achieved with 50 μg (0.11 μmoles) of the drug, as

compared with 150 μg (0.65 μmoles) of choline. This 6 fold difference in potency was somewhat surprising in view of the 50 fold difference in binding potencies (vs. [^{125}I]α-bungarotoxin) between the two drugs for the α7nAChR (Jonnala et al., 2003). However, Alkondon and colleagues (1999; 2000), in studying α7-mediated currents recorded from CA1 interneurons in rat hippocampus, reported that the EC50 values for choline and nicotine were, respectively, 2270 mM and 158 mM, representing only a 14 fold difference in potencies. Likewise there was no more than a 10 fold difference in potency between choline and nicotine in their ability to increase intracellular Ca^{2++} in cultured porcine superior cervical ganglion cells (Si and Lee, 2002). We had noted similar mismatches between binding affinity and pharmacological response earlier in studying nicotine and choline as cytoprotective agents in vitro (Jonnala et al., 2003), so it is not likely that the differences in drug effectiveness could be attributed to differences in their brain distribution or elimination. Also, choline appears to be slightly less efficacious than nicotine though it has been characterized as a full α7nAChR agonist (Pereira et al., 2002). Again, we had noted this potential discrepancy in our earlier cytoprotection study.

Although the effect of icv choline on heart rate was more variable than the effect on MAP, the responses appeared to be susceptible to the same antagonists as was the initial increase in MAP. MLA pretreatment almost completely normalized the biphasic change in heart rate to choline; whereas α-bungarotoxin pretreatment appeared to inhibit only the initial tachycardic phase. The differential effect may be due to differences in drug distribution between the two antagonists, and it suggests that the two phases of the heart rate response may be mediated in different brain regions. The ability of icv-injected atropine to inhibit the heart rate changes to choline suggests

that both muscarinic and nicotinic receptors play a role within the pathway mediating the bradycardic response to choline. The lack of effectiveness of atropine on the increase in blood pressure to choline indicates that the pressor pathway may not include muscarinic receptors.

This model not only provides a useful means for assessing the effectiveness of α7nAChR agonists and antagonists, but the results suggest that this subtype of nicotinic receptors plays some role in central cardiovascular regulation. For example, we noted earlier that central nicotinic receptors partly mediate the pressor responses to icv administration of certain neuro-active peptides such as bradykinin, angiotensin II, and substance P (Buccafusco and Serra, 1985; Trimarchi et al., 1986). More recently we reported that in rat models genetically manipulated to maintain an elevated blood pressure, there is a link between decreased expression of brain α7nAChRs and working memory deficits (Gattu et al., 1997; Terry et al., 2001). In the present study the pressor response to icv-administered choline was blocked by pretreatment with the α7 subtype selective antagonists, MLA and α-bungarotoxin. The lack of effect of pretreatment with DHβE, the β2 subtype-preferring antagonist, supported the concept that the initial pressor response to choline was mediated by the α7 subtype. Of course the participation of other non-α7 containing subtypes cannot be completely ruled out at this time; but the data do fit the known specificity of choline for α7nAChRs (Papke et al., 1996; Pereira et al., 2002; Jonnala et al., 2003). Additional support may be derived from the finding that an analog of choline, CDP-choline, also increased blood pressure and decreased heart rate after icv microinjection in rats (Savci et al., 2002). The pressor effect was significantly reduced following pretreatment with the non-selective nicotinic antagonist, mecamylamine.

Having characterized the specificity of the receptors mediating the pressor response to choline, it was then possible to determine whether icv administration of Aβ1-42 would inhibit the subsequent pressor response to icv-injected choline. Cerebroventricular infusion, or repeated icv injection of Aβ1-42 in rats has been used to assess the various pharmacological responses induced by the peptide, particularly regarding its potential amnestic actions (Maurice et al., 1996; Yamada et al., 1998; Yamaguchi et al., 2001; Nakamura et al., 2001; Olariu et al., 2002). Moreover, the related peptide Aβ25-35 (15 nmol/injection) has been reported to impair short-term working memory even after a single acute injection (Stepanichev et al., 2003). The dose used in the memory experiment was 150 fold greater than the most effective dose we used to block the pressor response to choline.

The mechanism by which Aβ1-42 interferes with the performance of certain memory-related tasks is not clear, but the peptide has this feature in common with the nicotinic receptor antagonist mecamylamine (Elrod and Buccafusco, 1991; Hiramatsu et al., 1998; Levin and Simon, 1998). Future studies will be required to determine whether the amnestic effects induced following central microinjection of Aβ peptides are related to their ability to bind to α7nAChRs. Nevertheless based on the data presented here, it is clear that Aβ1-42 does produce a functional blockade of α7nAChRs at low pharmacologic doses. The specificity of the Aβ1-42 inhibition of the choline pressor response was supported by the failure of Aβ40-1 peptide (which contains the critical α7nAChR binding sequence in reverse order) to block the choline response. Although Aβ1-42 significantly inhibited the pressor response to choline, the maximal degree of inhibition amounted to about a 60% decrease relative to the control response. Clearly the nicotinic antagonists were more effective, nearly eliminating the choline-induced pressor response. This

difference might reflect differences in the nature of the binding kinetics between Aβ1-42 and the antagonists. Alternatively, Aβ1-42 might not be distributed throughout the brain as readily as MLA and α-bungarotoxin. Indeed, the potential site of action for choline in eliciting its pressor response is not known. Stimulation of specific hypothalamic sites with cholinergic drugs has been reported to generate a pressor response mediated via nicotinic receptors (Schaeppi, 1967; Day and Roach, 1977). A hypothalamic site of action would explain the rapid onset of the pressor response to choline as the drug would readily redistribute to periventricular hypothalamic regions. To support a hypothalamic site of action, the pressor response to icv injection of CDP-choline was partly mediated via the release of vasopressin into the circulation (Savci et al., 2002). Also, α-bungarotoxin binding sites are relatively highly expressed in periventricular hypothalamic regions encompassing the dorsomedial, ventromedial, posterior hypothalamic nuclei, the dorsal premammilary nucleus and the arcuate nucleus (Gattu et al., 1997; Tohyama and Takatsuji, 1998).

In summary, these data support the concept that the pressor response to icv microinjection of choline may be used as an unbiased measure of α7nAChR stimulation *in vivo*. They also support the ability of acute injection of Aβ1-42 to produce a rapid functional blockade of α7nAChRs at pharmacologically (and likely physiologically in the case of AD) relevant doses. The use of telemetric catheters for monitoring cardiovascular status permits free movement of the animals and could allow for the assessment of various parameters of locomotor activity and other behaviors. Finally, some consideration should be given to the possibility that amyloid Aβ - α7nAChR interactions may play a role in the dysautonomia exhibited by some individuals with AD (Giubilei et al., 1998).

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Footnotes

This study was supported by the Office of Research and Development, Medical Research Service, Department of Veterans Affairs.

Figure Legends

Figure 1.

The time course for the change in mean arterial pressure (MAP) after icv injection of choline (50, 100, or 150 μg) in freely-moving rats. Each point and vertical bar represents the mean ± SEM derived from 4-6 experiments.

Figure 2.

The time course for the changes in heart rate after (HR) icv injection of choline (50, 100, or 150 μg) in freely-moving rats. Each point and vertical bar represents the mean ± SEM derived from 4-6 experiments.

Figure 3.

Effect of icv pretreatment with 10 μl of saline (vehicle), or with 10 μg of MLA, or with 0.3 μg of atropine on the change in MAP to icv injection of 150 μg of choline. Saline MLA, or atropine was injected 10 min prior to choline. Compared to saline, MLA significantly inhibited the pressor effect induced by choline [$F(1,497)=80.1$, $P<0.0001$]. Atropine pretreatment was without significant effect on the pressor response to choline [$F(1,497)=0.72$, $P=0.40$]. **Inset:** Inset: Accompanying HR changes to choline. Both MLA pretreatment [$F(1,497)=55.5$, $P<0.0001$] and atropine pretreatment [$F(1,495)=9.6$, $P=0.002$] inhibited the changes in HR to choline

Figure 4.

Effect of icv pretreatment with 10 μl of saline (control) or 10 μg of α-bungarotoxin (BTX), or 6 μg or dihydro-β-erythroidin (DHβE) on the change in MAP to icv injection of 150 μg of choline. Saline or the antagonists were injected 30 min prior to choline. When compared to the saline pretreatment group, α-bungarotoxin significantly inhibited the increase in MAP produced by 150 μg of choline [$F(1,465)=19.4$, $P<0.0001$]. Under the same conditions, 6 μg of DHβE failed to significantly alter choline induced pressor response [$F(1,350)=0.67$, $P=0.41$]. There also was a significant difference between the BTX and the DHβE treated groups [$F(1,350)=51.6$, $P<0.0001$]. Inset: Accompanying HR changes to choline. As with the effects on MAP, significant differences were found between the BTX vs. Saline pretreatment groups [$F(1,469)=34.2$, $P<0.0001$] and between the BTX vs. DHβE pretreatment groups [$F(1,350)=4.9$, $P=0.028$]. DHβE pretreatment failed to significantly inhibit the HR changes produced by choline [$F(1,350)=2.2$, $=0.14$].

Figure 5.

Dose effect of Aβ1-42 (1, 10, or 100 pmol, icv) on the choline (150 μg, icv)-induced changes in MAP. Amyloid peptides were administrated 30 min before choline. Aβ1-42 (100 pmol) significantly inhibited the increase in mean arterial pressure (MAP) produced by icv injection of choline [$F(1,645)=208.3$, $P<0.0001$]. The 10 pmol dose of Aβ1-42 also significantly inhibited the increase in MAP produced by choline relative both to the saline control [$F(1,492)=96.0$, $P<0.0001$]; and with respect to the 1 pmole dose [$F(1,492)=9.6$, $P=0.002$]. The 1 pmol dose of Aβ1-42 was not significantly effective in blocking the choline response ($P>0.05$). The first time point (0 min) indicates the time of choline administration**.**

Figure 6.

Comparison of the abilities of icv pretreatment with Aβ1-42 and the reverse sequence peptide Aβ40-1 to block the choline induced changes in MAP and heart rate. The peptides were administrated 30 min before choline. **Upper panel:** The pressor response to choline (150 μg, icv) was significantly greater following pretreatment with Aβ40-1 as compared with Aβ1-42 [$F(1,497)=33.3$, $P<0.0001$]; and Aβ40-1 pretreatment failed to significantly inhibit the pressor response to choline as compared to the data derived from vehicle pretreated rats [$F(1,405)=0.065$,$P=0.8$]. **Lower panel:** The changes in heart rate to choline were significantly greater following pretreatment with Aβ1-42 as compared with saline [$F(1,487)=46.7$, $P<0.0001$]; and Aβ40-1 pretreatment failed to significantly inhibit the changes in heart rate to choline as compared to the data derived from vehicle pretreated rats [$F(1,485)=0.58$, $P=0.4$].

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Table 1

Baseline values for Mean Arterial Pressure and Heart Rate for each study protocol.

	MAP (mmHg)	Heart Rate (beats/min)	N
Choline Dose Response (after saline pretreatment)			
50 μg	94±7.5	393±13.8	6
100 μg	108±5.8	384±16.1	6
150 μg	100±5.4	398±15.8	6
Treatments prior to Saline			
Saline	101±3.5	386±14.8	6
Treatments prior to Choline (150μg)			
MLA	99±7.4	359±18.3	6
BTX	105±8.7	392±15.8	6
Atropine	89±6.4	397±7.7	6
Aβ1-42 (100pmol)	96±4.2	385 ± 10.7	11
Aβ1-42 (10pmol)	98±5.4	394±13.6	6
Aβ1-42 (1pmol)	106±4.6	390±12.1	6
Aβ40-1 (100pmol)	90±3.4	385±8.3	6

Measurements were made during the 3-5 min preceding choline administration. MAP (mean

arterial pressure); MLA (methyllycaconitine); BTX (α-bungarotoxin). Each value represents the mean ± SEM. * P=0.005 as compared with saline (no injection) mean.

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Figure 1

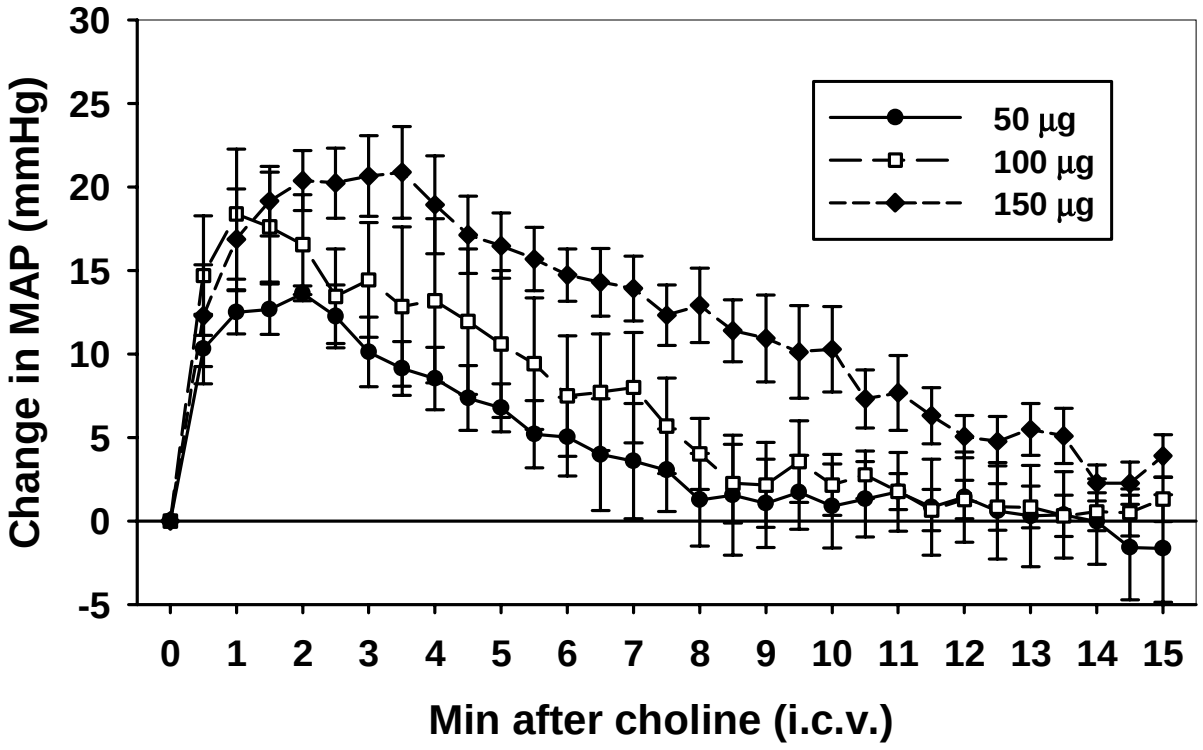

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Figure 2

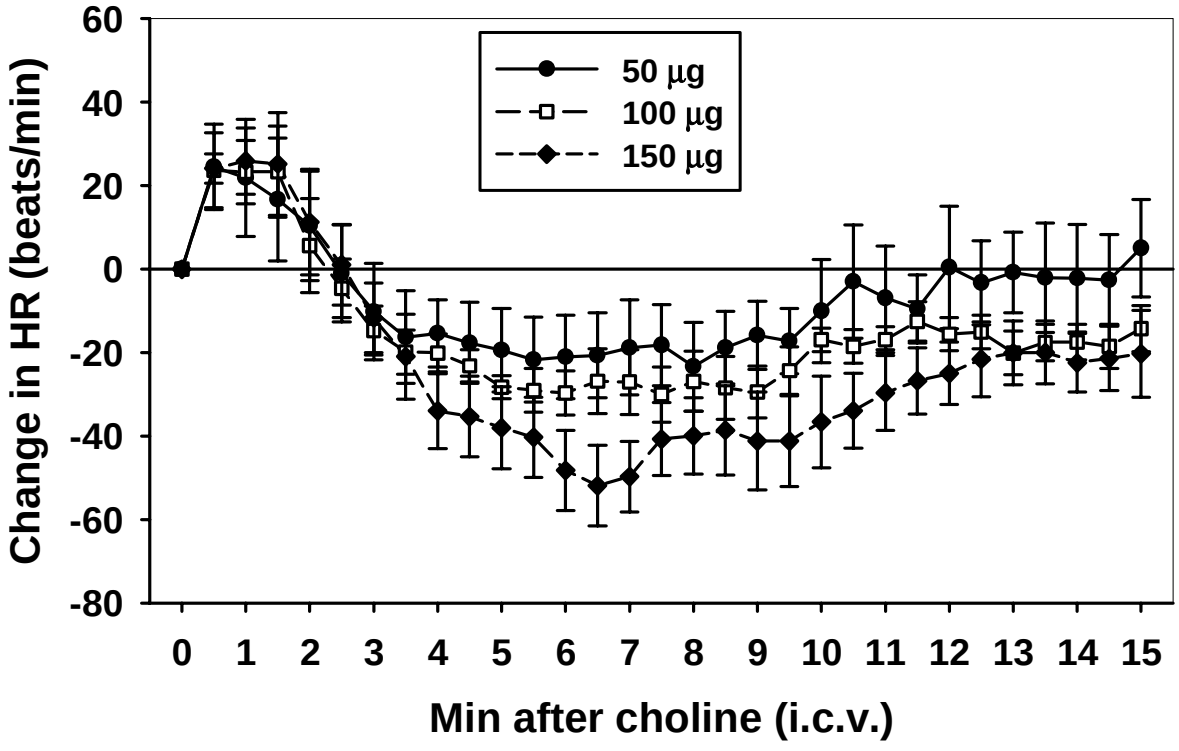

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Figure 3

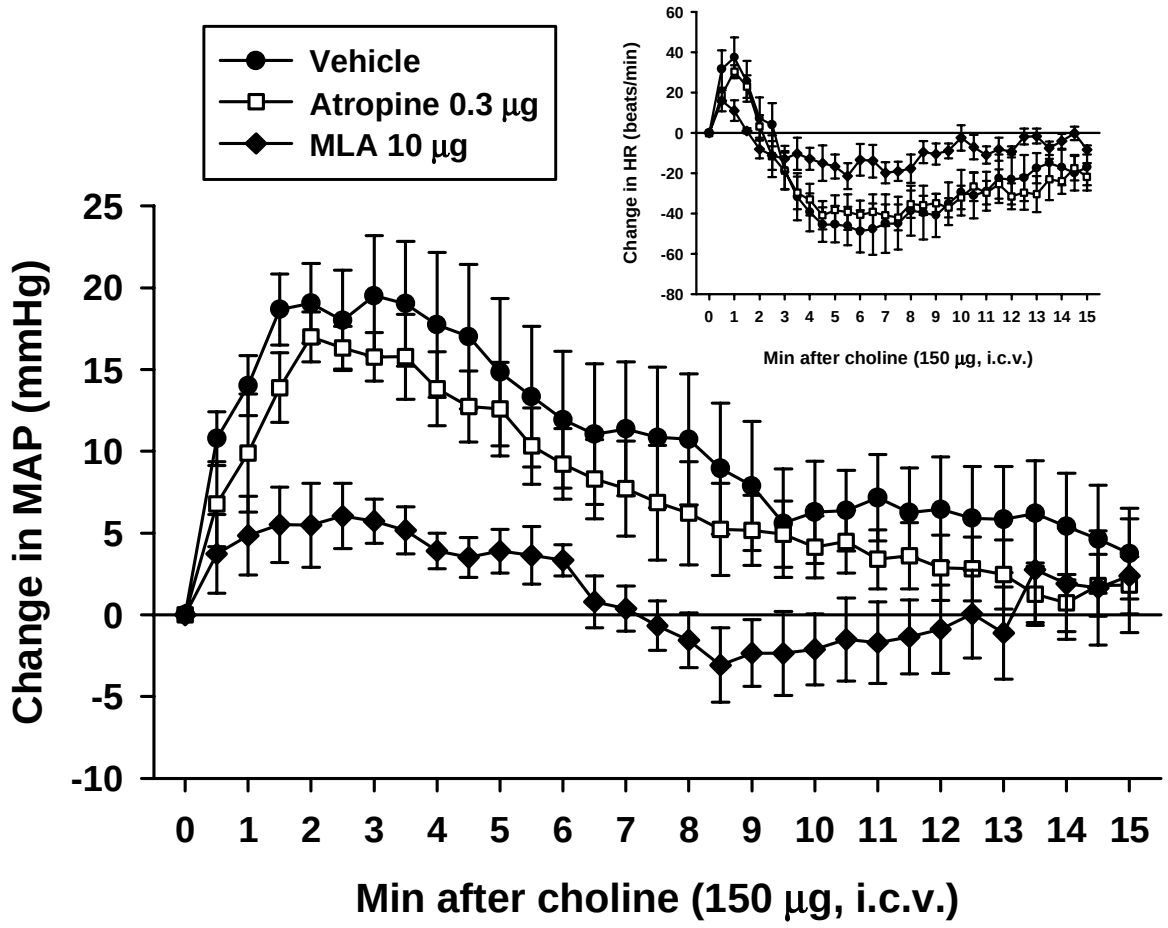

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Figure 4

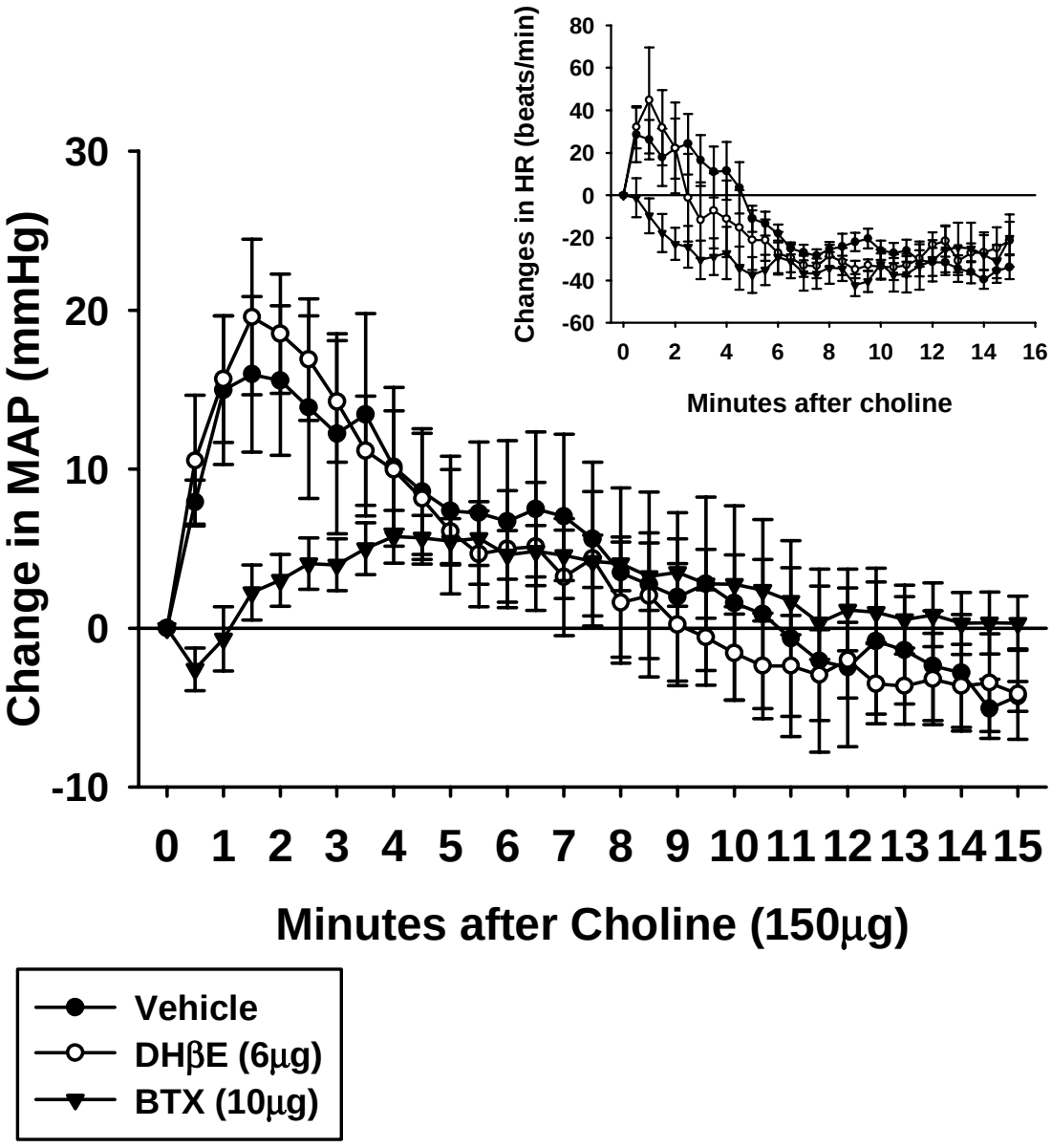

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Figure 5

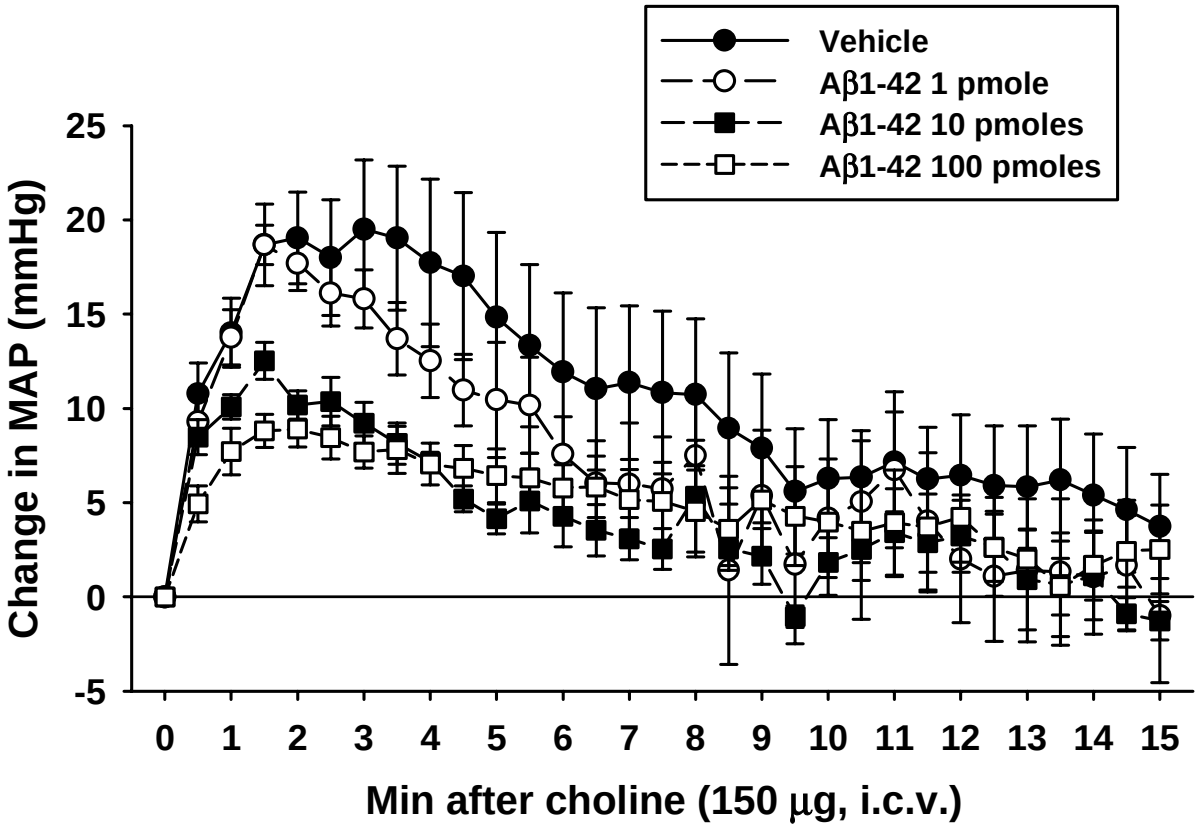

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Figure 6