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BRAIN RESEARCH

Research Report

# Endomorphin-2 in the medial NTS attenuates the responses to baroreflex activation

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ABSTRACT

We have previously reported that microinjections of endomorphin-2 (E-2; an endogenous mu-receptor agonist) into the medial subnucleus of the NTS (mNTS) elicit depressor and bradycardic responses via activation of ionotropic glutamate receptors located on secondary mNTS-neurons. Based on this report, it was hypothesized that activation of secondary mNTS neurons by E-2 may result in an exaggeration of baroreflex responses. In order to test this hypothesis, baroreflex responses were studied in adult, urethane-anesthetized, artificially ventilated, male Wistar rats before and after the microinjections of E-2 into the mNTS. Baroreceptors were stimulated by applying pressure increments (80–100 mm Hg) in the carotid sinus and by electrical stimulation (stimulus intensity: 0.5 V, frequencies 5, 10, and 25 pulses/s, pulse duration: 1 ms) of the aortic nerve for 30-s periods. Baroreceptor stimulation elicited depressor and bradycardic responses. Microinjections (100 nl) of E-2 (0.4 mmol/l) into the mNTS attenuated the baroreflex responses. Microinjections of naloxone (an opioid receptor antagonist) into the mNTS (0.5 mmol/l) did not alter baroreflex responses. Based on these results, it was concluded that activation of mu-opioid receptors in the mNTS attenuates baroreflex responses. Possible mechanisms for excitatory effects of E-2 in the mNTS resulting in depressor and bradycardic responses, on one hand, and inhibitory effects resulting in attenuation of baroreflex responses, on the other, are discussed.



## 1. Introduction

Baroreceptor afferents are known to make their primary synapse in the medial subnucleus of the nucleus tractus solitarius (mNTS) (Ciriello et al., 1994). Although there is a general consensus that an excitatory amino acid (probably glutamate) is the neurotransmitter released at the baroreceptor terminals in the NTS (Talman et al., 1984), other endogenous substances present in this nucleus may play a modulatory role in baroreflex. Endomorphins (two tetrapeptides, endomorphin-1 and -2), isolated from human and bovine brains, have been reported to possess a high affinity and selectivity for the mu-opioid receptors and are considered to be endogenous ligands for these receptors (Hackler et al., 1997; Zadina et al., 1997). The presence of endomorphin-like immunoreactivity (Martin-Schild et al., 1999; Pierce and Wessendorf, 2000) and opiate receptors (Atweh and Kuhar, 1977; Lord et al., 1977) in the mNTS has been demonstrated. We recently reported that microinjections of endomorphin-2 (E-2) into the mNTS elicited depressor and bradycardic responses (Kasamatsu et al., 2004). This result was unexpected considering that endomorphins are known to possess inhib-

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itory actions on neurons (Baraban et al., 1995; Dun et al., 2000; Guyenet et al., 2002; Hayar and Guyenet, 1998; Wu et al., 1999), and this effect is expected to elicit an increase in blood pressure (BP) and heart rate (HR) based on known circuitry of cardiovascular regulatory areas in the medulla (Dampney, 1994; Sapru, 2002). The depressor and bradycardic responses observed in our earlier study were mediated via activation of ionotropic glutamate receptors located on the secondary mNTS neurons (Kasamatsu et al., 2004). Based on this report (Kasamatsu et al., 2004), it was hypothesized that activation of secondary mNTS neurons by E-2 may result in an exaggeration of baroreflex responses. The present study was designed to test this hypothesis. However, the results showed that E-2 attenuated baroreflex responses. Possible mechanisms for excitatory effects of E-2 in the mNTS resulting in depressor and bradycardic responses, on one hand, and inhibitory effects resulting in attenuation of baroreflex responses, on the other, are discussed.

## 2. Results

### 2.1. E-2 attenuates the carotid sinus baroreflex

The concentration of E-2 (0.4 mmol/l) used in these experiments was selected on the basis of concentration-response studies carried out in one group of rats ($n$ = 7). In these concentration-response studies, the decreases in MAP elicited by 0.2, 0.4, and 0.8 mmol/l concentrations were 25 ± 1.4, 22.2 ± 2.6, and 17.2 ± 1.8 mm Hg, respectively. The decreases in HR elicited by the same concentrations of E-2 were 6.2 ± 2.3, 28.5 ± 3.5, and 18.5 ± 3.4 bpm, respectively. Microinjections of aCSF (100 nl) alone into the mNTS did not elicit any cardiovascular responses or alter baroreflex responses.

Microinjections of E-2 (0.4 mmol/l) into the mNTS attenuated the carotid sinus baroreflex responses. A typical tracing is shown in Fig. 1. The mNTS was always identified by microinjections of L-Glu, which stimulates neuronal cell bodies but not fibers of passage; microinjections of L-Glu (5 mmol/l) elicited typical decreases in MAP and HR (Fig. 1A). Five minutes later, mean carotid sinus pressure was increased from a basal level of 100 mm Hg to 200 mm Hg (i.e., a mean pressure increment of 100 mm Hg). The pressure increment was applied for 30 s; a decrease in MAP and HR resulted (Fig. 1B). Two minutes later, E-2 (0.4 mmol/l) was microinjected into the mNTS; depressor and bradycardic responses were elicited (Fig. 1C). E-2-induced decreases in BP and HR recovered within 5 min; repetition of the same pressure increment (i.e., 100 mm Hg) in the carotid sinus at this time elicited attenuated BP and HR responses (Fig. 1D). Recovery of the E-2-induced attenuation of carotid baroreflex was observed within 20 min (Fig. 1E). Group data ($n$ = 6) for this experiment are shown in Fig. 2. The decreases in MAP in response to a mean pressure increment of 80 mm Hg in the carotid sinus (i.e., the mean pressure was increased from the basal level of 100 mm Hg to 180 mm Hg) before and 5 min after the microinjection of E-2 (0.4 mmol/l) into the mNTS were 28 ± 3.1 and 10 ± 1.8 mm Hg, respectively ($P < 0.001$), and the baroreflex responses showed 80.3% recovery (i.e., 22.5 ± 1.7 mm Hg) within 20 min. Similarly, the decreases in

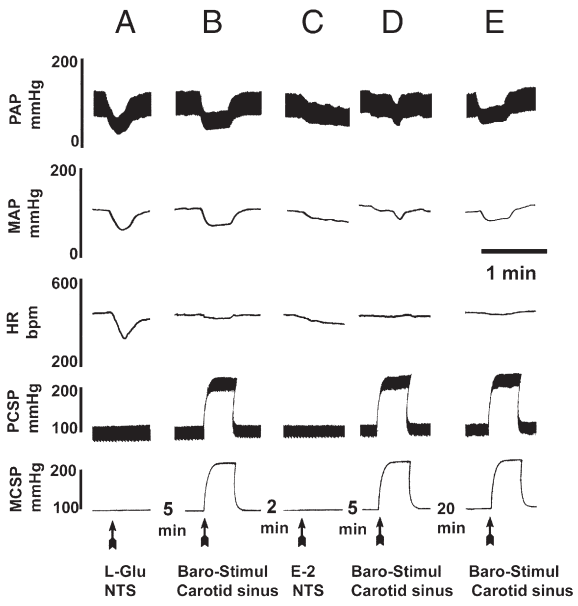


**Fig. 1 – Attenuation of carotid sinus baroreflex by endomorphin-2. Top trace: pulsatile arterial pressure (PAP, mm Hg), 2nd trace: mean arterial pressure (MAP, mm Hg), 3rd trace: HR (heart rate, bpm or beats/min), 4th trace: pulsatile carotid sinus pressure (PCSP, mm Hg), and 5th trace: mean carotid sinus pressure (MCSP, mm Hg). (A) mNTS was identified by a microinjection of L-Glu (5 mmol/l). (B) 5 min later, carotid sinus baroreceptors were stimulated by increasing mean pressure in the carotid sinus from a basal level of 100 mm Hg to 200 mm Hg (i.e., a 100 mm Hg mean pressure increment) for 30 s; reflex decrease in BP and HR was elicited. (C) 2 min later, endomorphin-2 (E-2; 100 nl, 0.4 mmol/l) was microinjected into the mNTS; depressor and bradycardic responses were elicited. (D) 5 min later, when the BP and HR returned to baseline, the responses to the same carotid sinus pressure stimulation were attenuated. (E) 20 min later, reflex BP and HR responses to carotid sinus baroreceptor stimulation recovered (about 70% and 42%, respectively).**

MAP in response to a mean pressure increment of 100 mm Hg in the carotid sinus before and 5 min after the microinjection of E-2 (0.4 mmol/l) into the mNTS were 32 ± 3.3 and 9.2 ± 1.5 mm Hg, respectively ($P < 0.001$), and the depressor responses showed 72.8% recovery (i.e., 23.3 ± 1.6 mm Hg) within 20 min (Fig. 2A). The decreases in HR in response to a mean pressure increment of 80 mm Hg in the carotid sinus before and 5 min after the microinjection of E-2 (0.4 mmol/l) into the mNTS were 21.6 ± 6.5 and 2.5 ± 2.5 bpm, respectively ($P < 0.05$), and these responses showed 34.7% recovery (i.e., 7.5 ± 4.7 bpm) within 20 min. The decreases in HR in response to a mean pressure increment of 100 mm Hg in the carotid sinus before and 5 min after the microinjection of E-2 (0.4 mmol/l) into the mNTS were 25.8 ± 3.7 and 5.8 ± 2 bpm, respectively ($P < 0.001$), and these responses showed 42.6% recovery (i.e., 11 ± 0.6 bpm) within 20 min (Fig. 2B). Lesser mean pressure increments in the carotid sinus (e.g., 40 and 60 mm Hg) were not applied in this experiment because the reflex responses were not robust at these pressure increases.