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Long-lasting inhibition of the cardiovascular responses to glutamate and the baroreceptor reflex elicited by neuropeptide Y injected into the nucleus tractus solitarius of the rat

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Neuropeptide Y (NPY) microinjected unilaterally into the nucleus tractus solitarii (NTS) of anesthetized paralyzed rats elicits a gradual dose-dependent and reversible fall in arterial pressure (AP) and heart rate (HR) lasting 20 min. It also abolished the brief (< 1 min) dose-dependent and reversible fall of AP and HR elicited by L-glutamate (L-Glu) injected into the nucleus. The blockade of L-Glu by NPY appeared gradually and was prolonged, lasting over 2 h, and recovering by 24 h. It was not replicated by desamido-NPY or galanin. Unlike 2% lidocaine it did not block the hypotension elicited by focal electrical stimulation at the injection site indicating the response was not that of a local anesthetic. Bilateral injection of NPY into the NTS resulted, after an initial fall, in an elevation of AP ($+48 \pm 10.6$ mmHg). At this time the reflex bradycardia evoked by elevating AP with phenylephrine was markedly reduced. We conclude that in the NTS, NPY antagonizes the actions of L-Glu and may attenuate baroreceptor reflexes. Since the NTS is richly innervated by NPY neurons and contains many NPY binding sites and since primary baroreceptor afferents appear to be glutamatergic the results suggested that NPY may serve in NTS as a long-term regulator of baroreceptor reflex activity.

The nucleus tractus solitarii (NTS), the site of termination of primary afferent fibers of arterial baroreceptors, is richly innervated by neurons containing neuropeptide Y (NPY) [6, 10]. That NPY may have a function within the NTS is supported by the findings that the nucleus contains many NPY binding sites [8, 11, 16] and that microinjection of NPY into NTS lowers arterial pressure (AP) and heart rate (HR) [2, 4, 18]. One mechanism by which NPY might act within the NTS would be by interaction with L-glutamate (L-Glu), the presumed endogenous transmitter of primary baroreceptor afferents [12, 17]. In the present study, we therefore sought to determine whether NPY microinjected into the NTS modifies the cardiovascular response to local administration of L-Glu. We report that NPY will elicit a long-lasting inhibition of the responses to L-Glu within the NTS and also substantially inhibits the reflex bradycardia elicited by acutely elevating AP pharmacologically.

Studies were conducted on male Sprague–Dawley rats (300–360 g). The animals were anesthetized, after induction with isoflurane (1.5% in 100% O_2), with urethane (1 g/kg, i.p.) (Sigma, MO, U.S.A.) and α-chloralose (35 mg/kg, i.p.) (Fisher Sci. Co., NJ, U.S.A.). Cannulas were inserted in one femoral artery and vein. The trachea was cannulated, the rats paralyzed with tubocurarine (0.12 mg/kg, i.m.) and ventilated on 100% O_2. Injection of chloralose (1.5 mg) and tubocurarine (0.03 mg/kg) were repeated hourly. Body temperature was maintained at 37°C with a thermostatically controlled heating pad. AP, mean arterial pressure (MAP) and HR were recorded from the femoral catheter and displayed on channels of a chart recorder.

Animals were then placed in a stereotactic frame (Kopf) with the bite bar set at 12 mm below the intraaural line, the head flexed downward at an angle of 45° and the brainstem exposed through a small craniotomy. The calamus scriptorius served as the stereotactic zero. Agents were microinjected into a site of mediolateral NTS whose stereotactic coordinates (dorsal angle of 15°) were 0.3 mm rostral, 0.7 mm lateral, and 0.5 mm ventral to zero.

Agents were microinjected into NTS through glass pipettes with an external tip diameter of 40–50 μm. Materials were delivered slowly by a microinfusion pump

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(L-Glu, 30 nl over 8–10 s; peptide or vehicle, 90 nl over 25–30 s). All agents were dissolved in artificial cerebrospinal fluid of the following composition (g): NaCl 7.21, $NaHCO_3$ 1.97, KCl 0.18, KH_2PO_4 0.068, $CaCl_2$ 0.162, $MgCl_2+6H_2O$ 0.169, Na_2SO_4 0.071, glucose 1.06 dissolved in 1 liter redistilled water. The cardiovascularly active site in NTS was functionally identified by injection of L-Glu (0.7 nmol) a dose that evoked 80% of maximal fall in AP and HR.

The NTS was electrically (cathodally) stimulated through electrodes fabricated from teflon-coated stainless steel wire with an external tip diameter of 10μm. Square wave pulses were generated by a stimulator and delivered to the electrode via constant-current stimulus isolation unit. The anode consisted of a clip attached to the neck muscles. At the termination of an experiment microinjection sites were marked by replacing the cannula with one containing Black Magic ink (Faber-Castell, NJ, U.S.A.) or an electrode through which a small lesion was made by passage of a DC current (0.5 mA, 30 s). The rats were killed by i.a. infusion of saturated KCl. The brains were removed and rapidly frozen to −70°C. They were subsequently sectioned on a cryostat and stained with thionin for localization of cannula tips or electrode sites.

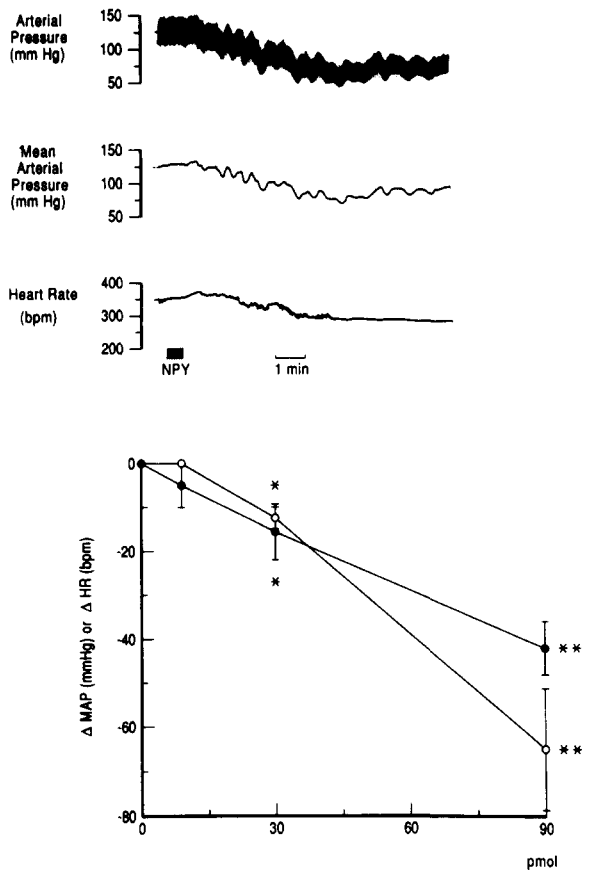

Fig. 1. Upper panel: effects of NPY (90 pmol in 90 nl) injected into NTS upon pulsatile AP, mean AP and HR. In this and all subsequent figures, studies are performed in anesthetized paralyzed rats. Note the delay in onset, the gradual decline and marked variability of AP response. Lower panel: relationship between the fall in MAP (closed circles) and HR (open circles) and dose of NPY injected unilaterally into NTS. Each dose given to a naive rat. AP and HR before injection were: 0=vehicle 133.0±8.4 mmHg, 338.±19.7 bpm; NPY (9 pmol) 96.7±3.8 mmHg, 360±26.4 bpm; NPY (30 pmol) 119.2±8.6 mmHg, 352.5±26.3 bpm and NPY (90 pmol) 122.0±2.2 mmHg, 352.5±12.2 bpm, $*P<0.05$, $**P<0.01$ ($n=4$–8 for each group).

L-Glu and phenylephrine hydrochloride was purchased from Sigma, MO, U.S.A.; rat NPY and rat galanin from Bachem Inc., CA, U.S.A.; desamido-NPY from Peninsula, CA, U.S.A.

The results are expressed as mean values ±S.E.M. Multiple comparisons were analyzed using the analysis of variance and the Newman-Keuls test. Single differences between groups were evaluated using two-tailed unpaired Student's t-test. Differences were considered significant for $P<0.05$.

Effects of NPY in NTS. NPY (9–90 pmol) injected unilaterally into the NTS elicited dose-dependent reductions in AP and HR (Fig. 1). The fall in AP evoked by a maximal dose of NPY (90 pmol) appeared after a delay of many seconds, declined gradually to a nadir by 5 to 10 min, and returned to baseline usually within 30 min. The AP following injection of NPY exhibited a marked variability. The fall in HR, also characterized by variability, paralleled in onset the change in AP. However, it often persisted for at least 1 h (Fig. 1) before recovering to baseline.

To determine whether the cardiovascular response to NPY was chemically selective, we examined the effects upon AP and HR of microinjection into NTS of desamido-NPY or galanin. Desamido-NPY exerts virtually no biological activity in various tissues [7, 20, 21]. Galanin, a neuropeptide also contained in NTS [9, 15] mimics several effects of NPY, e.g. inhibiting the release of NE in the hypothalamus [19].

Desamido-NPY had no effect on AP or HR when injected into NTS ($n=6$). Galanin (90 pmol) did not affect AP but induced a fall in HR of −30±6.4 beats per min (bpm) ($P<0.05$ from baseline; $n=4$); HR returned to baseline within 10–15 min.

Effects of L-Glu in NTS. L-Glu injected into NTS, in agreement with earlier reports [17], induced a marked dose-dependent transient fall in AP and HR (Fig. 2). In contrast to the responses to NPY, the fall of AP and HR appeared immediately and without variability, reached a nadir within 10–15 s, and rapidly recovered together within 60 s.

Interaction of NPY and L-Glu in NTS. To determine whether NPY could modify the response to L-Glu, NPY (90 pmol) was injected unilaterally into the NTS. Ten

min later and at varying times thereafter, L-Glu at a dose which was submaximal for the vasodepressor response (0.7 nmol), was injected into the same site.

Pre-treatment with NPY, but not vehicle, progressively inhibited and then abolished the fall in HR and AP elicited by L-Glu over 10–20 min (Fig. 3). Thereafter, for the remainder of the experiment (1–3 h), L-Glu, even at the maximal effective dose (3 nmol, not shown), had no effects.

To estimate the duration of the NPY-evoked inhibition of the L-Glu responses, 4 rats were anesthetized with isoflurane (2%), and NPY (90 pmol) was injected unilaterally into NTS and vehicle (90 nl) into the contralateral NTS. The wounds were closed, anesthesia discontinued and the animals returned to their cages. Twenty-four hours later they were reanesthetized, instrumented (see Methods), the NTS exposed and L-Glu (0.7 nmol) injected into the NTS. L-Glu injected into the NPY-treated side of NTS induced reductions in AP and HR of -50.0 ± 2.4 mmHg and -97.5 ± 18.3 bpm, respectively, which did not differ ($P>0.05$) from the responses (-48.5 ± 2.1 mmHg, and -76.2 ± 12.5 bpm) evoked contralaterally. Thus, the NPY-evoked inhibition of L-Glu responses was reversed within 24 h.

The effect of NPY on the response to L-Glu was topographically and chemically selective. Contralateral injection of L-Glu (0.7 nmol) into NTS at 25 min after NPY treatment evoked cardiovascular responses (-59.6 ± 8.0 mmHg, -86.2 ± 5.5 bpm) which were the same as the responses prior to NPY treatment (-64.6 ± 4.1 mmHg, -95.0 ± 24.0 bpm; $n=4$, n.s.). Desamido-NPY (90 pmol; $n=6$) and galanin (90 pmol; $n=4$) had no effect on the cardiovascular responses induced by L-Glu.

To assess whether NPY could be acting in the NTS as a local anesthetic we compared the effects of locally mic-

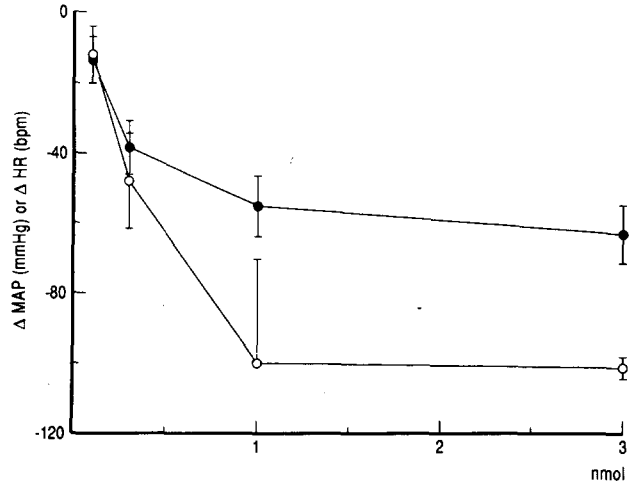

Fig. 2. Relationship between the fall of AP (●) and HR (○) and cumulative dose of L-Glu microinjected (90 nl) into NTS. Baseline values of MAP and HR were 116.7 ± 3.6 mmHg, 358.2 ± 10.8 bpm ($n=4$).

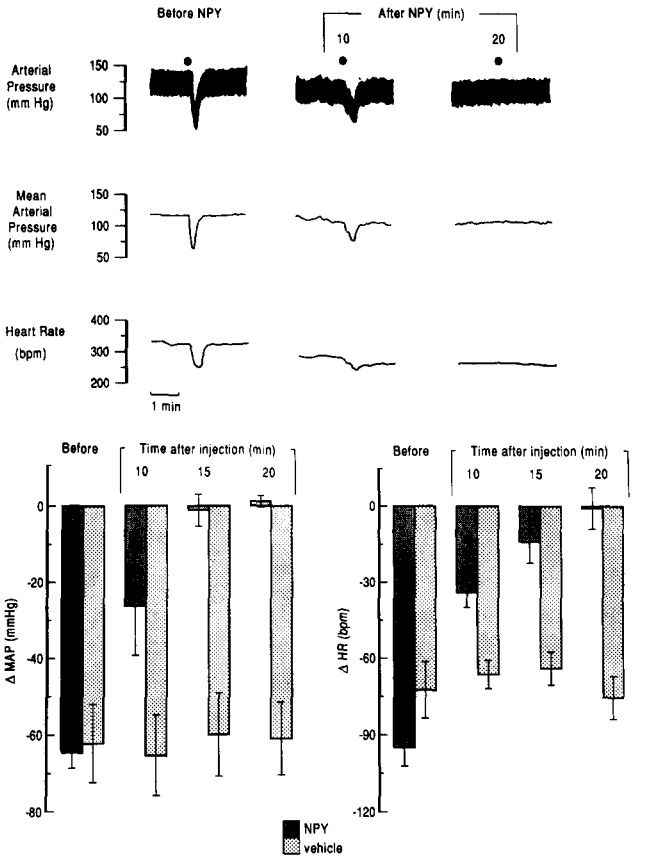

Fig. 3. Upper panel: effects of NPY (90 pmol in 90 nl) microinjected unilaterally into NTS on the responses of AP and HR to microinjection of L-Glu (●) (0.7 nmol). Tracings show responses to L-Glu before and at 10 and 20 min after injection of NPY. Note the rapid onset and recovery of cardiovascular responses to L-Glu in comparison to NPY (Fig. 1). Lower panel: comparisons over time of the reductions of MAP (left panel), and HR (right panel) elicited by microinjection of L-Glu (0.7 nmol) at the same site into the NTS before, 10, 15 and 20 min after injection of NPY (90 pmol, $n=8$) (dark bars), or vehicle (90 nl, $n=4$) (stippled bars). Note the response to L-Glu is unaffected in vehicle treated side while the onset is not complete until 20 min in the NPY treated side.

roinjected NPY or 2% lidocaine into the NTS upon the hypotension elicited by focal electrical stimulation. NPY (90 pmol in 90 nl), vehicle (90 nl) or lidocaine (2%; 90 nl) were injected unilaterally into each side of NTS in a series of rats. An electrode was placed into the same side and stimulated 25–30 min later. Electrical stimulation (20 Hz, 0.5 ms, 40 μA and 3 s) of NTS resulted in a comparable fall in AP and HR in the NPY-treated (-45.7 ± 4.9 mmHg, -65.0 ± 10.2 bpm, $n=4$) and vehicle-treated sides (-45.0 ± 6.1 mmHg, -57.5 ± 16.5 bpm). However, injection of lidocaine into the vehicle-treated side totally abolished the electrically stimulated cardiovascular responses for about 10–15 min indicating that NPY does not act as a local anesthetic.

Effects of NPY upon baroreflexes. Finally, we examined the effects of bilateral microinjection of NPY into the

NTS on resting AP and HR and the reflex bradycardia elicited by elevation of systemic AP with phenylephrine.

In 5 untreated rats, phenylephrine (10 μg/kg, i.v.) increased MAP from basal levels (112±17.0 mmHg) by +63.7±9.4 mmHg and simultaneously reduced HR (basal, 352±26.6 bpm) by −68.7±15.6 bpm.

In 4 rats, NPY was bilaterally injected into the NTS (90 pmol in each side). NPY resulted, within 5 min, in an initial decrease in AP by −37±4.5 mmHg from basal levels of 105±11.0 mmHg and a fall in HR by −74±17.5 bpm from a baseline of 318±25.8 bpm. AP and HR thereafter reversed and by 25 min, AP increased +48.0±10.6 mmHg from baseline without change in HR (−2±18.8 bpm). The elevation in AP was sustained for about 30 min.

In these rats, at a time when AP and HR were recovering to basal levels (118.7±13.3 mmHg and 318.7±26.8 bpm) phenylephrine was administered as before. Phenylephrine elevated AP by +57.5±8.3 mmHg, an elevation comparable to controls. However, the reflex fall in HR was reduced to −20±4.6 bpm ($P<0.05$ from untreated control). These observations suggest that NPY not only blocks the response to L-Glu but also inhibits the cardiac limb of the baroreflex.

The present study indicates that NPY microinjected into the NTS has two different effects upon AP. The first is a dose-dependent and reversible fall in AP and HR, a finding in general accord with observations of others [2, 4, 18]. However, a feature of the response not reported elsewhere is the slow onset, gradual decline, and marked variability of AP, the latter a feature associated with interference with baroreflex mechanisms peripherally or centrally [1, 3, 13, 14].

The second response, not heretofore reported, was inhibition by NPY of the vasodepressor responses to microinjection into the same site of L-Glu. The inhibition of the response to L-Glu was prolonged, potent, and reversible persisting more than 2 and less than 24 h. The onset of inhibition of L-Glu was gradual and not complete until approximately 20 min after administration of the peptide, a time when the hypotensive response to NPY had recovered. The response was site-specific and cannot be attributed to a local anesthetic effect of the peptide since, unlike lidocaine, it did not block the cardiovascular responses to focal electrical stimulation at the injection site. Taken together with the observations that desamido-NPY and galanin were ineffective in inhibiting the L-Glu-induced responses those results suggest that the NPY effect is receptor-mediated and exhibits biochemical selectivity.

NPY locally microinjected into NTS not only blocked the effect of exogenous L-Glu but also appeared to interfere with arterial baroreceptor reflexes. Thus, when NPY was injected bilaterally into the nucleus, after an initial phase of hypotension, AP rose and the reflex bradycardia elicited by acutely elevating AP with phenylephrine was significantly attenuated, effects comparable to those elicited by interrupting baroreceptor activity centrally or peripherally [1, 3, 13, 14]. The facts that NPY blocks the responses to exogenous L-Glu and attenuates baroreflex activity is consistent with the evidence that L-Glu is the transmitter of baroreflex afferents [12, 17] and suggests that the exogenously applied NPY blocks synaptic actions of L-Glu released from endogenous sources. The observations that NPY inhibits the release of L-Glu in hippocampal slices [5] indicates that an interaction of NPY with L-Glu may occur elsewhere in brain, however, probably by different mechanisms.

The finding that NPY initially stimulated and then impaired baroreceptor reflexes as well as the cardiovascular responses to exogenous L-Glu is very similar to the responses elicited by microinjection of long-lasting L-Glu agonist kainic acid into the NTS [17]. In the case of kainate the response has been attributed to an initial stimulation followed by depolarization blockade of neurons distal to baroreceptor afferents within the nucleus. Whether comparable mechanisms account for the bimodal action of NPY within the NTS is unknown.

The findings, therefore, further demonstrate the possibility that NPY which is contained within nerve terminals within the NTS may act to regulate cardiovascular function [6, 9, 10, 18]. However, the finding that its actions are prolonged raises the prospect that it may not act in the second by second regulation of cardiovascular activity. Rather, it may contribute to long term adaptive processes in the NTS conceivably by adjusting set-points within the cardiovascular reflex networks integrated there.

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