Dexamethasone attenuates the depressor response induced by neuropeptide Y microinjected into the nucleus tractus solitarius in rats

[1]Ming Ouyang & *,[1]Shao Wang

[1]Department of Physiology, Norman Bethune University of Medical Sciences, Changchun 130021, Jilin, China

1 An investigation was made of the effect of dexamethasone (Dex) injection into the nucleus tractus solitarius (NTS) on the cardiovascular response to neuropeptide Y in rats.

2 Dex (39 pmol) injected into the NTS inhibited the hypotension and bradycardia caused by NPY (5 pmol) with a short latency (10 min) and a long duration of action (up to 4 h).

3 The rapid inhibition by Dex (39 pmol) of the cardiovascular response to NPY was not blocked by pretreatment with the glucocorticoid receptor blocker, RU38486 (47 or 117 pmol respectively), but was reversed by bicuculline (30 pmol).

4 Microiontophoresis of NPY (0.01 mM, pH 6.5) into the NTS increased the spontaneous firing of the majority (68.4%) of baroreflex-excited cells, but decreased the firing of most (73.7%) baroreflex-inhibited cells. In contrast, Dex (0.02 M, pH 6.5) decreased the spontaneous firing of the majority of baroreflex-excited cells (42.1% of normal response) and decreased the inhibition of baroreflex-inhibited cells (47.5% of normal response). The responses of the majority of baroreceptive cells to NPY were blocked by iontophoretic administration of Dex.

5 Dex (200 μM) increased the delayed rectifier outward K + current by $31.4 \pm 1.1\%$ ($n = 5$), whereas NPY alone, at a concentration of 1.5 μM, inhibited the current by $28.6 \pm 0.8\%$ ($n = 5$). In the presence of Dex (200 μM), addition of NPY (1.5 μM) had no effect on the current.

6 In conclusion, NTS-administered-Dex attenuated the cardiovascular response to NPY injected into the same area *via* a rapid membrane effect, which was mediated by an action on $GABA_A$ receptors and on the delayed rectifier outward K^+ channel.

British Journal of Pharmacology (2000) **129**, 865–870

Introduction

It is well known that glucocorticoids participate in the regulation of blood pressure (Yagil *et al.*, 1986; Tonolo *et al.*, 1988; Grunfeld & Eloy, 1987). Glucocorticoid receptor (GR) immunoreactivity was found in adrenergic neurons and neuropeptide Y neurons in the nucleus tractus solitarius (NTS) (Fuxe *et al.*, 1986; Harfstrand *et al.*, 1986; 1989). Since NTS neurons are the termination points for all afferent baroreceptor fibres and have α_2-adrenoceptors and neuropeptide Y (NPY) receptors (Yang *et al.*, 1994), they play an important role in cardiovascular regulation (Kalia, 1981; Andresen & Kunze, 1994), and direct application of noradrenaline (NA) or NPY to the NTS has potent effects on the cardiovascular system (De Jong, 1974; Tseng *et al.*, 1988). The depressor and bradycardia responses to direct application of noradrenaline to the NTS are abolished by prior administration of dexamethasone (Dex) (Ouyang *et al.*, 1999). It is very likely that the cardiovascular responses to NPY application may also be influenced by glucocorticoids. There-fore, the present study was designed to test the effects of dexamethasone (Dex) on the cardiovascular response to NPY microinjected into the NTS and to analyse the underlying mechanism of its actions.

Methods

Cardiovascular experiments

Adult Wistar rats of either sex (250–300 g) were anaesthetized with a mixture of α-chloralose (35 mg kg^{-1}, i.p.) and urethane (1 g kg^{-1}, i.p.). The trachea was cannulated to prevent airway obstruction. A heparinized catheter (50 u ml^{-1} in 0.9% w v^{-1} saline) was inserted into the femoral artery and connected to a pressure transducer attached to a polygraph recorder for monitoring blood pressure and heart rate. The animal was placed on a heating pad to maintain rectal temperature at $37 \pm 0.5°C$. The head of the animal was placed in a stereotaxic frame and adjusted to 45° from the horizontal plane. Neck muscles were electrocauterized to expose the posterior altano-

*Author for correspondence; E-mail: wangsh@public.cc.jl.cn

occipital membrane and fine dissection was used to expose the caudal medulla in the region of the obex and calamus scriptorium.

Unilateral microinjections were made stereotaxically into the NTS with a glass micropipette (tip diameter 50–60) connected to a microsyringe by polyethylene tubing. The tip of the pipette was positioned 0.5 mm rostral to the obex, 0.5 mm lateral to midline and 0.6 mm deep to the surface of brainstem. Dexamethasone-21-phosphate disodium, NPY and bicuculline (Bic) were dissolved in 0.9% saline (pH 7.2), whereas RU38486, a glucocorticoid receptor antagonist, was dissolved in sesame oil. Both Dex and RU38486 bind to the membrane-bound glucocorticoid receptor (Chen *et al.*, 1993; Quelle *et al.*, 1988) but the binding-affinity of RU38486 is greater than that of Dex (Chen *et al.*, 1993). RU38486 also has a high affinity for the cytosolic glucocorticoid receptor and is a reliable competitive antagonist of this receptor (Agarwal *et al.*, 1987). This compound has no agonist activity at either receptor. Each drug solution was delivered in a volume of 0.1 μl over approximately 20 s. Saline and sesame oil was used for control injections.

Experiment 1: NPY (5 pmol) was microinjected into the NTS, either alone or at various intervals (10 min, 0.5, 1, 2, 3 and 4 h) after Dex (39 pmol). Dex alone did not evoke any change in cardiovascular function.

Experiment 2: In order to determine whether the effect of Dex on the NPY response was mediated by glucocorticoid receptors, Dex was injected into the NTS 10 min after administration of the GR antagonist, RU38486 (47 or 117 pmol). Then, NPY (5 pmol) was locally applied to the same area, 30 min after injection of Dex. Microinjection of the GR antagonist alone had no effect on cardiovascular function.

Experiment 3: NPY (5 pmol) was administered into the NTS 10 min after coinjection of Dex (39 pmol) and Bic (30 pmol), a blocker of GABA$_A$ receptors. Microinjection of Bic alone had no effect on cardiovascular function (Barron *et al.*, 1997).

Basal values of blood pressure were recorded for at least 15 min before each microinjection. At the conclusion of the experiment, 0.1 μl pontamine sky blue was injected for histological verification of injection sites. Data are expressed as mean $\pm$ s.e.mean. Statistical significance between experimental and control groups was assessed with ANOVA, followed by Dunnett *t*-tests where appropriate.

Microiontophoresis studies

Methods for recording blood pressure and exposing the caudal medulla were the same as for the cardiovascular experiments. An additional catheter was inserted into the femoral vein for infusion of phenylphrine (2–4 kg^{-1}, pH 7.4), in order to activate the baroreflex. Body temperature was maintained at 37 $\pm$ 0.5°C *via* a heating blanket. Blood pressure and heart rate were monitored on a polygraph recorder.

Five-barreled glass microelectrodes with 2–5 μm tips were used to record extracellularly from single neuron in the NTS at the level of the area postrema, and to apply drugs by microiontophoresis at the site of recording. The central barrel was filled with a 2% solution (w v^{-1}) of pontamine sky blue in 0.5 M sodium acetate and was used for recording cellular activity. Recording barrel resistance typically ranged from 7–12 MΩ. Three of the peripheral barrels were filled with the following drugs: NPY (0.01 mM, pH 6.5), anti-serum raised against NPY (dilution 1:500, pH 6.0), and Dex (0.02 M, pH 6.5). Automatic current balance was maintained through a fourth peripheral barrel containing 4 M NaCl. Positive and

negative currents were passed through this barrel to control the possible current artifacts that might arise during drug application. NPY and NPY antiserum were iontophoresed as cations (using currents of up to 80 nA), whereas Dex was ionophoresed as anions (currents of up to 90 nA) or the otherwise was retained with backing currents of 15 nA.

A microdrive manipulator was used to advance or reverse the recording electrodes. When cells were encountered, their discharges were displayed on an oscilloscope (VC-10, Nihon Koden). Baseline firing rates were measured for 3 min of spontaneous activity. Baroreceptive cells were identified by infusion of phenylephrine through the femoral vein, and then drugs were applied by iontophoresis over 1 min. Neuronal discharges were recorded on videotape with a Sony videocassette recorder. After a cell was fully tested, the position of the recording tract was marked by passing 20 μA of anionic current through the recording barrel for 30 min, to deposit pontamine sky blue at the electrode tip. Animals were then perfused intracardially with 10% v v^{-1} formalin. The locations of each recording track and tested neurons were histologically verified in 50 frozen sections.

Neuronal discharges were analysed by IBM-compatible PC computer and histograms were constructed by HP-Laser 4LC Printer. Neurons were categorized as inhibited or excited, depending on whether iontophoretic application of drug produced a reproducible change in firing rate that was equal to or greater than 50% of baseline (measured for 30 s prior to drug application). Neurons, which failed to meet this criterion, were classified as unresponsive. The statistical significance between experimental groups was assessed by X^2-analysis for unpaired data.

Whole-cell recordings

Wistar rats (12–15 day-old) were killed by cervical dislocation. The brainstem was rapidly removed and placed in cold (4°C) buffer (in mM): NaCl 110, sodium succinate 10, KCl 5, CaCl$_2$ 0.2, MgCl$_2$ 5, D-glucose 15, HEPES 15, pH 7.4, and equilibrated with 100% O$_2$. Using a vibratome, a horizontal medullary slice (700 μm) was prepared from the area containing the medial and dorsal solitary nuclei, 0.5 mm rostral and 2 mm caudal to the obex. The slices were equilibrated in HEPES buffer with 100% O$_2$ at 32°C for 2 h. They were then placed in buffer containing pronase E (1 mg ml^{-1}) for 40 min at 32°C. The neurons were isolated by gentle trituration in DMEM culture medium, using fire-polished Pasteur pipettes of decreasing diameter. The whole solution was then transferred to a recording dish. The neurons were allowed to settle on the bottom and the DMEM replaced with an extracellular solution comprising (in mM): NaCl 137, KCl 5.4, MgCl$_2$ 1, D-glucose 10, TTX 0.01, 4-aminopyridine 5. Whole cell recordings were obtained from neurons that were characteristically bipolar in shape, and typically 15.0 $\times$ 10.5 3.03 m in size. This type of neuron has been previously shown to receive baroreceptor terminals (Mendelowitz *et al.*, 1992).

Methods for making patch pipettes and whole cell recording were similar to those described by Hamill *et al.* (1981). The patch pipettes had a resistance of 3 MΩ when tested in extracellular solution. Pipettes were filled with a solution containing (in mM) KCl 140, MgCl$_2$ 2, Na$_2$ATP 2, EGTA 10, HEPES 10, and titrated to pH 7.3. The indifferent electrode was an Ag-AgCl plug connected to the bath *via* a 150 mM KCl agar bridge. Voltage-activated currents were evoked using the 0.3203 Ap CLAMP 0.3203 A program (Version 6.0, Axon Instruments), run on an IBM compatible microcomputer DT 125 kHz interface (Lab Master). To obtain outward potassium

currents, the cell was clamped at a holding potential of -80 mV, and serially depolarized in $+10$ mV steps from -80 mV to $+30$ mV, each sustained 300 ms in duration. Voltage-activated currents were also analysed and plotted using CLAMP program. Data are expressed as mean $\pm$ s.e.mean. The statistical significance between experimental groups was assessed by Student t-test for paired data. All recordings were made at 22.5°C.

Drugs

All of the following substances were obtained from Sigma: dexamethasone-21-phosphate disodium (Dex), neuropeptide Y (NPY), neuropeptide Y antiserum, bicuculline (Bic), phenylephrine, pontamine sky blue, pronase E, HEPES, ethyleneglycolbis- (b-aminoethyl-ether)-N,N,N N tetraacetic acid (EGTA), tetraethyl-ammonium chloride (TEA), 4-aminopyridine (4-AP), tetrodotoxin (TTX), Na_2ATP (adenosine-5 triphosphate disodium salt), and DMEM (Dulbecco Modified Eagle Medium). RU38486 [7β-hydroxy-11β-(4-dimethylamino-phenyl) 17α-(1-propymyl) estra-4,9-diene-3-one] was kindly donated by Roussel-UCLAF (Romainville, France).

Results

Effect of Dex on the depressor response induced by NPY

As seen in Table 1, NPY (5 pmol) alone injected into the NTS significantly decreased both blood pressure and heart rate (-29.0 ± 2.7 mmHg, -35 ± 11 beats min^{-1}, $n = 12$). Microinjection of Dex (39 pmol) into the NTS did not significantly alter the hypotensive and bradycardic responses to simultaneous administration of NPY (5 pmol). However, if Dex was injected 10 min before NPY, it markedly attenuated the depressor and bradycardic responses to NPY, which effect remained for 4 h (Figures 1 and 2).
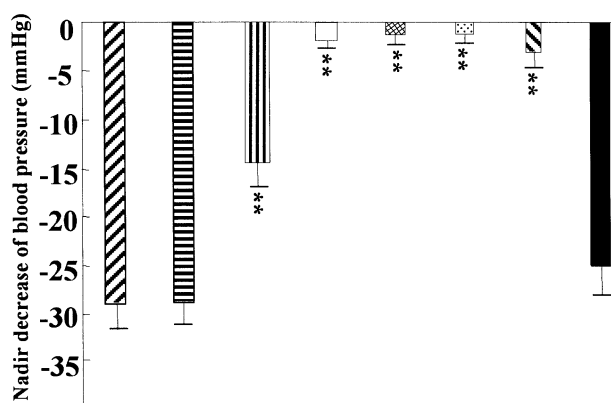

Figure 1 Effect of Dex on changes of blood pressure induced by microinjection of NPY into the NTS. NPY (5 pmol) alone microinjected into the NTS; NPY (5 pmol) and Dex (20 ng) coinjected into the NTS; NPY (5 pmol) microinjected into the NTS 10 min after pretreatment with Dex (20 ng); NPY (5 pmol) microinjected into the NTS 30 min after pretreatment with Dex (20 ng); NPY (5 pmol) microinjected into the NTS 60 min after pretreatment with Dex (20 ng); NPY (5 pmol) microinjected into the NTS 120 min after pretreatment with Dex (20 ng); NPY (5 pmol) microinjected into the NTS 180 min after pretreatment with Dex (20 ng); NPY (5 pmol) microinjected into the NTS 240 min after pretreatment with Dex (20 ng).
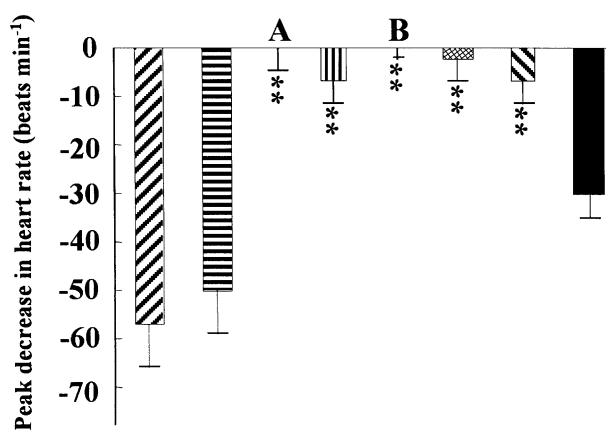

Figure 2 Effect of Dex on changes of heart rate induced by microinjection of NPY into the NTS. NPY (5 pmol) alone injected into the NTS; NPY (5 pmol) and Dex (20 ng) coinjected into the NTS; A: NPY (5 pmol) microinjected into the NTS 10 min after Dex (20 ng) administered into the same area; NPY (5 pmol) microinjected into the NTS 30 min after Dex (20 ng) administered into the same area; B: NPY (5 pmol) microinjected into the NTS 60 min after Dex (20 ng) administered into the same area; NPY (5 pmol) microinjected into the NTS 120 min after Dex (20 ng) administered into the same area; NPY (5 pmol) microinjected into the NTS 180 min after Dex (20 ng) administered into the same area; NPY (5 pmol) microinjected into the NTS 240 min after Dex (20 ng) administered into the same area.

In vivo electrophysiological data

Seventy-five neurons located in the NTS were examined for their responses to transient elevations of arterial blood pressure. A neuron was classified as baroreceptive if it displayed either an increase or decrease of at least 30% from baseline spike activity in response to transient elevation of blood pressure. Figure 3 illustrates such responses observed in 38 of the 75 cells (50.7%) after infusion of phenylephrine. Of these 38 baroreceptive cells, 19 displayed decreases in firing rates, whereas the remaining 19 showed the increases. All of the baroreceptive cells were tested for their responses to iontophoretic administration of Dex, NPY and NPY antiserum. Iontophoresis of Dex made the spontaneous firing of some baroreflex-excited cells decrease (42.1%) and those of some baroreflex-inhibited cells increase (47.5%). In contrast, application of NPY ($n = 34$) increased the spontaneous firing of the majority of baroreflex-excited cells (68.4%) and decreased the firing of baroreflex-inhibited cells (73.7%) (Figure 4). And all the responses to NPY were blocked by local administration of NPY antiserum (data not shown). If the treatment of Dex before the administration of NPY would attenuate both of these responses in the majority of baroreceptive cells (73.5% cells, $P < 0.01$) (Figure 4).

Mechanism of the Dex effect on NPY responses

The results described above show that Dex has a rapid effect on the hypotensive responses to NPY. In order to test whether this effect involves the GR, the GR antagonist RU38486 was used. Local application of RU38486 (47 or 117 pmol) to the NTS 10 min before Dex did not block the rapid inhibitory effect of Dex on the hypotensive response to NTS-administered-NPY (Table 2), suggesting that the effects of Dex are not mediated by the GR.

Because corticosterone, the natural glucocorticoid, produces rapid effects in the nervous system by modulating $GABA_A$ receptors (Gee, 1988; Turner et al., 1989), we then

Table 1 Effect of microinjection of Dex into the NTS on cardiovascular response to NTS-administered-NPY

| | Arterial blood pressure | | Heart rate | |
Treatment	Basal value (mmHg)	Peak decrease (mmHg)	Basal value (beats min^{-1})	Peak decrease (beats min^{-1})
NPY alone	102.8 ± 2.0	-29.0 ± 2.7	360 ± 10	-57 ± 9
Dex group				
0 min	102.0 ± 2.0	-28.8 ± 2.4	364 ± 10	-50 ± 9
10 min	102.0 ± 2.1	-14.4 ± 2.6**	360 ± 17	0 ± 0.9**
30 min	102.8 ± 2.0	-2.0 ± 0.8**	361 ± 16	-8 ± 5**
60 min	102.8 ± 2.6	-1.5 ± 1.0**	365 ± 12	0 ± 2**
120 min	101.0 ± 2.2	-1.0 ± 0.8**	364 ± 10	-3 ± 2**
180 min	102.8 ± 2.7	-3.0 ± 1.8**	365 ± 17	-5 ± 5**
240 min	100.0 ± 2.2	-25.0 ± 3.1	363 ± 11	-30 ± 5

Values were expressed as means±s.e.mean for $n = 12 \sim 15$. The peak effects were expressed maximal decrease amplitude from the respective basal value. Minutes in Dex group meant various time intervals when NPY (5 pmol) was microinjected into the NTS after NTS administered-Dex (20 ng). **$P < 0.01$ compared with NPY alone group analysing by ANOVA followed by Dunn test.
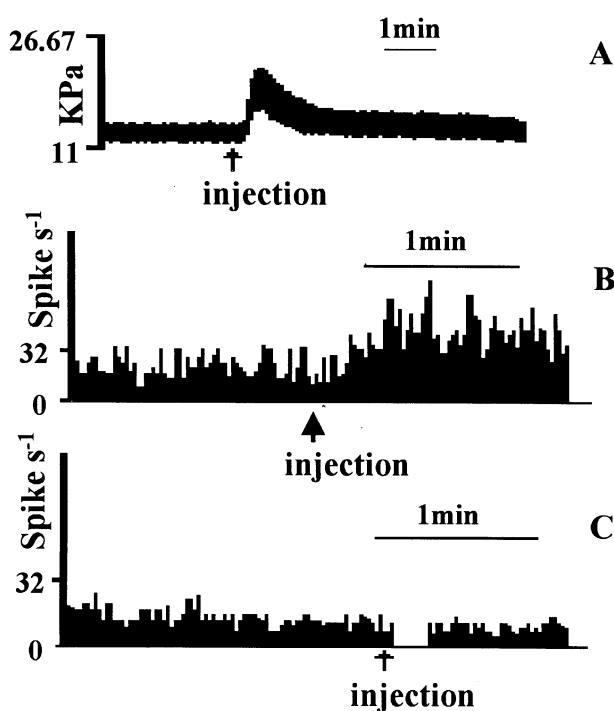

Figure 3 Baroreceptive cells in the NTS response to arterial blood pressure elevated. (A) Arterial blood pressure changed after injection of phenylephrine; (B) Baroreflex-excited cell; (C) Baroreflex-inhibited cell.
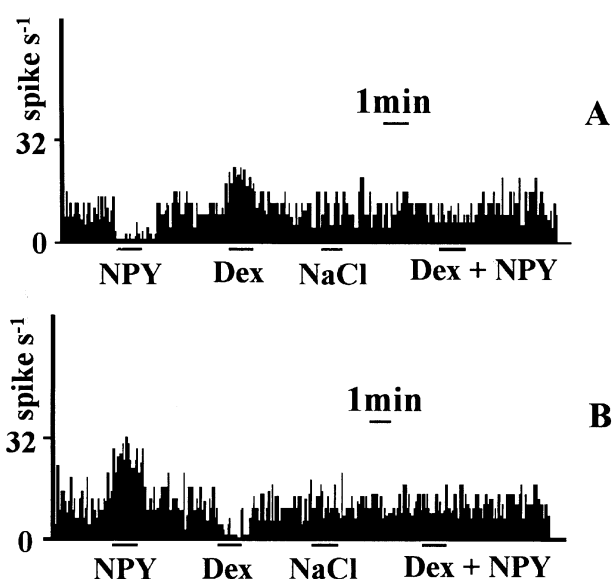

Figure 4 Effects of NPY and Dex on the activities of baroreceptive cells in the NTS. (A) Baroreflex-inhibited cell; (B) Baroreflex-excited cell.

examined this possibility in the NTS. Bic (a GABA$_A$ receptor antagonist; 30 pmol) was administered simultaneously with Dex (39 pmol), 30 min before administration of NPY (5 pmol). The inhibitory effect of Dex on NPY responses was abolished by Bic (Table 3).

In vitro *electrophysiological data*

With 0.01 mM TTX and 5 mM 4-AP present in the extracellular solution, and nominally Ca^{2+}-free internal and external solutions, a delayed rectifier outward K$^+$ current was evident when the membrane potential was held at -80 mV and serially depolarized in $+10$ mV steps from -80 mV to $+30$ mV (Figure 4). This current was incompletely blocked by 10 mM TEA. The current was activated slowly and the time to reach half of the peak current ($\tau 1/2$) was voltage dependent (25.6 ± 2.6 ms at 0 mV,

20.8 ± 2.2 ms at $+10$ mV, 15.2 ± 1.2 ms at $+20$ mV, 10.5 ± 1.0 at $+30$ mV; $n = 4$). The delayed rectifier outward K$^+$ current was not activated if the potential was less than -30 mV (Figure 5).

Application of Dex (200 μM) increased the delayed rectifier outward K$^+$ current by $31.4 \pm 1.1\%$ ($n = 5$). NPY alone (1.5 μM) inhibited the current by $28.6 \pm 0.8\%$ ($n = 5$), but in the presence of Dex (200 μM), NPY had no effect (Figure 6).

Discussion

These results demonstrate that microinjection of Dex into the NTS attenuates the hypotension and bradycardia induced by NPY administered into the same area. The genomic effect of steroid hormones takes longer than 30 min to occur (typically hours to days). However, the inhibitory effect of Dex in the NTS occurred rapidly, within 10 min. Our microiontophoretic data provides further evidence for the rapid effect of Dex in the NTS. Within just 1 min Dex not only attenuated the effects of NPY on baroreceptive cells, but also in many cases abolished the NPY effects.

Table 2 Effect of glucocorticoid receptor on the rapid inhibition of Dex on the cardiovascular response to NTS-administered-NPY

Treatment	Arterial blood pressure		Heart rate	
	Basal value (mmHg)	Peak decrease (mmHg)	Basal value (beats min^{-1})	Peak decrease (beats min^{-1})
Control A	104.3 ± 3.0	-30.1 ± 3.0	360 ± 11	-55 ± 10
Control B	103.8 ± 3.2	-1.0 ± 0.8	360 ± 14	-10 ± 8
Experiment A	102.9 ± 2.6	-2.0 ± 1.4	370 ± 16	-5 ± 5
Experiment B	103.5 ± 3.4	-3.5 ± 2.2	365 ± 15	-10 ± 6

Values were expressed as means $\pm$ s.e.mean for $n = 15 \sim 15$. The peak effects were expressed maximal decrease amplitude from the respective basal value. Microinjection of Dex (20 ng) into the NTS 10 min after pretreatment with RU38486 (20 ng, experiment A; or 50 ng, experiment B) in the experiment group (or sesame oil in the control B). And NPY (5 pmol) was applied into the same area 30 min after injection of Dex (control B, experiment). In control A, NPY was microinjected into the NTS 30 min after injection of sesame oil. **$P < 0.01$ vs control B analysing by ANOVA followed by Dunn test.

Table 3 Effect of GABA$_A$ receptor on the rapid inhibition of Dex on the cardiovascular response to NTS-administered-NPY

Treatment	Arterial blood pressure		Heart rate	
	Basal value (mmHg)	Peak decrease (mmHg)	Basal value (beats min^{-1})	Peak decrease (beats min^{-1})
Control	103.1 ± 3.2	-3.5 ± 1.7	375 ± 15	-8 ± 5
Experiment	102.6 ± 2.9	$-18.7 \pm 1.4**$	370 ± 14	$-50 \pm 11**$

Values were expressed as means $\pm$ s.e.mean for $n = 15 \sim 15$. The peak effects were expressed maximal decrease amplitude from the respective basal value. Microinjection of NPY (5 pmol) into the NTS 30 min after coinjection of Dex (20 ng) with Bic (15 ng, or saline in the control) in the experiment group. **$P < 0.01$ compared with control group by unpaired student t-test.
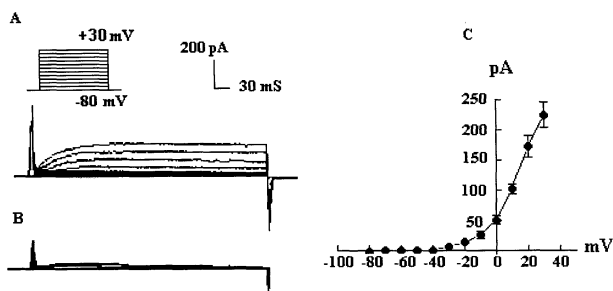

Figure 5 Delayed rectifier outward potassium current. (A) Current records in response to depolarization in steps from -80 to $+30$ mV; (B) Current records under addition of TEA (10 mM); (C) Current-voltage relation of steady state delayed rectifier outward potassium current for five cells.

Figure 6 Effects of Dex and NPY on the delayed rectifier outward potassium currents of acutely dissociated neuron in the NTS. (A) Current records in response to depolarization in steps from -80 to $+30$ mV under normal extracellular solution; (B) Current response to addition of Dex (200 μM) into the extracellular solution; (C) Current response to addition of NPY (1.5 μM) into the extracellular solution; (D) Current response to addition of NPY (1.5 μM) into the extracellular solution after pretreatment with Dex (200 μM).

There are several ways in which steroids can have rapid actions on membranes (Golden et al., 1998; Orchinik et al., 1991; Passaquin et al., 1998; Quelle et al., 1998; Sandi et al., 1996; Turner et al., 1989). One mechanism is the allosteric modulation of the GABA$_A$ receptor (Gee, 1988; Turner et al., 1989). In the present study, the glucocorticoid receptor antagonist, RU38486, in concentrations sufficient to block the effect of Dex on glucocorticoid receptors (Zhu et al., 1995), did not block the rapid inhibition by Dex of the cardiovascular response to NTS-administered-NPY. However, injection of Bic into the NTS, which does not alter blood pressure and heart rate by itself (Barron et al., 1997), prevented the Dex effect. NPY neurons in the NTS not only express glucocorticoid receptors (Harfstrand et al., 1989), but also receive inputs from GABAergic terminals (Pickel et al., 1989). Our results suggest that Dex may influence these neurons by modulating their GABA$_A$ receptor activity. Activation of GABA$_A$ receptors in the NTS tonically elevates blood pressure and decreases the depressor reflex (Kubo & Kihara, 1988; Barron et al., 1997). This is consistent with the effect of Dex on the
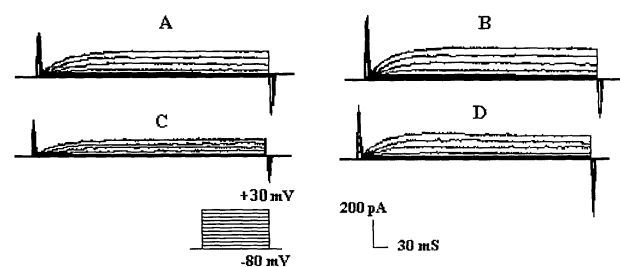
activity of baroreceptive NTS cells in our study and indicates that the decreases in depressor reflex are likely to be mediated by GABA$_A$ receptors rather than the glucocorticoid receptor. Whether the mechanism of this effect on the GABA$_A$ receptor is the same as endogenous corticosterone is yet to be determined.

There is also evidence that glucocorticoids modulate various plasma membrane ion channels to reduce responses of excitable cells (Joels & de Kloet, 1994; Wang, 1997). For example, Dex modulates the release of ACTH through changes in activity of Na$^+$-channels, Ca^{2+}-activated K$^+$-channels and the delayed rectifier outward K$^+$-channel (Lim et al., 1998; Shipston et al., 1996). In the present study, we recorded a delayed rectifier outward K$^+$ current in NTS neurons, using Ca^{2+}-free internal and external solutions with the whole cell recording configuration. Dex augmented this current in the NTS and decreased membrane excitability. In contrast, NPY attenuated the delayed rectifier current, which increased

membrane excitability. This may be an important mechanism underlying the rapid inhibitory effect of Dex on the cardiovascular response to NPY.

In conclusion, microinjection of Dex into the NTS attenuates the cardiovascular response to NTS-administered NPY through rapid actions on membrane. The rapid inhibitory effect of Dex in the NTS was probably due to a combined effect on modulation of the GABA$_A$ receptor and the delayed rectifier outward K$^+$ channel, rather than *via* the glucocorticoid receptor. Dex also has a rapid inhibitory effect on the cardiovascular response to NA microinjected into the NTS (Ouyang *et al.*, 1999), and this may occur through a similar mechanism.

References

AGARWAL, M.K., HAINQUE, R., MOUSTAID, N. & LAZER, G. (1987). Glucocorticoid antagonists. *FEBS Lett.*, **217**, 221 – 225.

ANDRESEN, M.C. & KUNZE, D.L. (1994). Nucleus tractus solitarius gateway to neural circulatory control. *Ann. Rev. Physiol.*, **56**, 93 – 116.

BARRON, K.W., PAVELKA, S.M. & GARRETT, K.M. (1997). Diazepan-sensitive GABA$_A$ receptors in the NTS participate in cardiovascular control. *Brain Res.*, **773**, 53 – 60.

CHEN, Y.Z., FU, H. & GUO, Z. (1993). Membrane receptor for glucocorticoids in mammalian Neurons. In: Conn, M. (ed), *Methods in Neurosciences.* **Vol. 11,** Academic Press, San Diego. pp. 16 – 28.

DE JONG, W. (1974). Noradrenaline: central inhibitory control of blood pressure and heart rate. *Eur. J. Pharmacol.*, **29**, 179 – 181.

FUXE, K., AGNATI, L.F., HARFSTRAND, A., JASON, A.M., NEU-MEYER, A., NDESSON, K., RUGGERI, M., ZOLI, M. & GOLD-STEIN, M. (1986). Morphofunctional studies on the neuropeptide Y/adrenaline costoring nerve terminal systems in the dorsal cardiovascular region of the medulla oblongata. Focus on receptor-receptor interactions in cotransmission. *Prog. Brain Res.*, **68**, 303 – 320.

GEE, K. (1988). Steroid modulation of the GABA/benzodiazene receptor-linked chloride ionophore. *Mol. Neurobiol.*, **2**, 201 – 217.

GOLDEN, G.A., RUBIN, R.T. & MASON, R.P. (1998). Steroid hormones partition to distinct sites in a model membrane bilayer: direct demonstration by small-angle X-Ray diffraction. *Biochimica et Biophysica Acta*, **1368**, 161 – 166.

GRUNFELD, J.P. & ELOY, L. (1987). Role des glucocorticoids dans la regulation de la pression arterielle. *Press Med.*, **16**, 1365 – 1367.

HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.*, **329**, 85 – 100.

HARFSTRAND, A., CINTRA, A., FUXE, K., ARONSSON, M., WIK-STROM, A. & OKRET, S. (1989). Regional differences in glucocorticoid receptor immunoreactivity among neuropeptide Y immunoreactive neurons of the rat brain. *Acta Physiol. Scand.*, **135**, 3 – 9.

HARFSTRAND, A., FUXE, K., CINTRA, A., AGNATI, L.F., ZINI, I., WIKSTROM, A.-C., OKRET, S., YU, Z.-Y., GOLDSTEIN, M., STEINBUSCH, H., VERHOFSTRAD, A. & GUSTAFSSON, J.-A. (1986). Glucocorticoid receptor immunoreactivity in monaminergic neurons of rat brain. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 9779 – 9783.

JOELS, S.M. & DE KLOET, E.R. (1994). Mineralocorticoid and glucocorticoid receptors in the brain – implications for ion permeability and transmitter systems. *Prog. Neurobiol.*, **43**, 1 – 36.

KALIA, M.P. (1981). Localization of aortic and carotid baroreceptor and chemoreceptor primary afferents in the brain stem. In: Ferrario, C.M. (ed.) *Central Nervous System Mechanism in Hypertension.* Raven Press: New York. pp. 9 – 24.

KUBO, T. & KIHARA, M. (1988). Evidence for γ-aminobutyric acid receptor-mediated modulation of the aortic baroreceptor reflex in the nucleus tractus solitarii of the rat. *Neurosci. Lett.*, **89**, 156 – 160.

LIM, M.C., SHIPSTON, M.J. & ANTONI, F.A. (1998). Depolarization counteracts glucocorticoid inhibition of adenohypophysical corticotroph cells. *Br. J. Pharmacol.*, **124**, 1735 – 1743.

MENDELOWITZ, D., YANG, M., ANDRESEN, M.C. & KUNZE, D.L. (1992). Localization and retention in vitro of fluorescently aortic baroreceptor terminals on neurons from nucleus tractus solitarius. *Brain Res.*, **581**, 339 – 343.

ORCHINIK, M., MURRAY, T.F. & MOORE, F.L. (1991). A cortisosteroid receptor in neuronal membranes. *Science*, **252**, 1848 – 1851.

OUYANG, M., GE, G.H. & WANG, S. (1999). Effect of dexamethasone on cardiovascular response induced by noradrenaline in the nucleus tractus solitarius. *Acta Pharmacol. Sin.*, **20**, 167 – 170.

OUYANG, M. & WANG, S. (1999). Rapid vasodepression induced by large dose of dexamethasone injected into the nucleus tractus solitarius. *Acta Zool. Sin.*, **45**, 311 – 316.

PASSAQUIN, A.-C., LHOTE, P. & RUEGG, U.T. (1998). Calcium influx inhibition by steroids and analogs in C2C12 skeletal muscle cells. *Br. J. Pharmacol.*, **124**, 1751 – 1759.

PICKEL, V.M., CHAN, J. & MASSARI, V.J. (1989). Neuropeptide Y-like immunoreactivity in neurons of the solitary tract nuclei: vesicular localization and synaptic input from GABAergic terminals. *Brain Res.*, **476**, 265 – 278.

QUELLE, F.W., SMITH, R.V., HRYCYNA, C.A., KALIBAN, T.D., CROOKS, J.A. & O RIEN, J.M. (1988). [³H]Dexamethasone binding to plasma membrane-enriched fractions from liver of nonadrenalectomized rats. *Endocrinology*, **123**, 1642 – 1651.

SANDI, C., VENERO, C. & GUAZA, C. (1996). Nitric Oxide synthesis inhibitors prevent Rapid behavioral effects of corcoticosterone in rats. *Neuroendocrinology*, **63**, 446 – 453.

SHIPSTON, M.J., KELLY, J.S. & ANTONI, F.A. (1996). Glucocorticoids block protein kinase A inhibition of calcium-activated potassium channels. *J. Biol. Chem.*, **271**, 9197 – 9200.

TONOLO, G., FRASER, R., CONNEL, J.H.C. & KENYON, C.J. (1988). Chronic low-dose infusion of dexamethasone in rats: Effects on blood pressure, body weight and plasma atrial netriuretic peptide. *J. Hypertens.*, **6**, 25 – 31.

TSENG, C.H., MOSQUEDA-GRACIA, R., APPALSAMY, M. & ROBERTSON, D. (1988). Cardiovascular effects of neuropeptide Y in rat brain stem nuclei. *Circ. Res.*, **64**, 55 – 61.

TURNER, D.M., RANSON, R.W., YANG, J.S. & OLSEN, R.W. (1989). Steroid anesthetic and naturally occurring analogs modulate the GABA$_A$ receptor complex at a site distinct from barbiturates. *J. Pharmacol. Exp. Ther.*, **248**, 960 – 966.

WANG, W. (1997). The ionic mechanism of hyperpolarization induced by glucocorticoid and its modulatory effects in mammalian neurons. *Prog. Physiol. Sci.*, **28**, 229 – 231.

YAGIL, Y., KOREEN, R. & KRAKOFF, L.R. (1986). Role of mineralocorticoids and glucocorticoids in blood pressure regulation in normotensive rats. *Am. J. Physiol.*, **251**, H1354 – H1360.

YANG, S.-N., FIOR, D.F., HEDLUND, P.B., AGNATI, L.F. & FUXE, K. (1994). Antagonistic regulation of α$_2$-adrenoceptors by neuropeptide Y receptor subtypes in the nuclear tractus solitarii. *Eur. J. Pharmacol.*, **271**, 201 – 212.

ZHU, B.G. & CHEN, Y.Z. (1995). Rapid facilitation of glucocorticoids on the high affinity uptake of L-glutamine by cerebral synapses in rats. *Chin. J. Neurosci.*, (suppl): P156.