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Differences in cardiovascular responses to peripherally administered GABA as influenced by basal conditions and type of anaesthesia

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1 The cardiovascular (blood pressure, heart rate, cardiac contractility) effects of i.v. γ-aminobutyric acid (GABA) were investigated in guinea-pigs anaesthetized with barbitone or urethane.

2 GABA (0.1–10 mg kg^{-1}) produced a transient 'depressive' effect on cardiovascular parameters which in barbitone-anaesthetized animals was followed by a transient 'excitatory' effect. Resting cardiovascular parameters were higher in urethane- as compared to barbitone- anaesthetized animals.

3 Picrotoxin pretreatment (2 mg kg^{-1}, i.v.) barely affected the cardiovascular changes produced by GABA in barbitone-anaesthetized animals. In picrotoxin pretreated animals anaesthetized with urethane, GABA produced an initial depression of cardiovascular parameters followed by an excitatory phase.

4 Hexamethonium (20 mg kg^{-1}, i.v) suppressed or reduced markedly the GABA-induced cardiovascular changes both in barbitone- or urethane- anaesthetized animals.

5 Reserpine pretreatment lowered resting cardiovascular parameters. In these animals, regardless of type of anaesthesia, the effects of i.v. GABA were of the 'excitatory' type only. Reserpine pretreated animals anaesthetized with barbitone were selected for further experiments.

6 Various $GABA_A$ receptor agonists (homotaurine, muscimol, THIP, 5-aminovaleric acid) mimicked the 'excitatory' effect of GABA in reserpine pretreated animals anaesthetized with barbitone and prevented the effects of subsequent GABA administration. On the other hand (±)-baclofen, a selective $GABA_B$ receptor agonist, had a slight depressant effect and did not prevent the 'excitatory' cardiovascular effects of GABA.

7 Neither bicuculline nor picrotoxin pretreatment prevented the 'excitatory' cardiovascular effect of i.v. GABA in reserpine pretreated, guinea-pigs anaesthetized with barbitone.

8 In adrenalectomized guinea-pigs or in preparations receiving i.v. phentolamine plus propranolol, GABA produced only a small 'depressant' effect on cardiovascular parameters.

9 These findings demonstrate that GABA exerts a neuromodulatory effect on cardiovascular function via peripheral actions which is influenced by: (a) type of anaesthesia (b) resting values of cardiovascular parameters (c) degree of activity of the sympathetic nervous system and (d) catecholamine release from the adrenal medulla.

Introduction

Earlier studies indicated that, in anaesthetized animals, intravenous (i.v.) γ-aminobutyric (GABA) produces transient cardiovascular changes, usually of the depressive type (i.e. hypotension and bradycardia) (Takahashi *et al.*, 1955; 1958; Elliott & Hobbiger, 1959; Stanton & Woodhouse, 1960; Stanton, 1963), although in some instances pressor/depressor episodes were observed (Stanton & Woodhouse, 1960).

Intensity, duration and even characteristics of cardiovascular changes produced by i.v. GABA vary

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significantly from species to species and, sometimes, within the same species. Depth of anaesthesia (Takahashi *et al.*, 1958; Elliott & Hobbiger, 1959; Stanton & Woodhouse, 1960) and also the use of different anaesthetics (barbiturates, chloralose, urethane) may have played a role in the genesis of this variability, making it difficult to compare results from different studies.

The aim of the present study was to evaluate the cardiovascular effect of i.v. GABA in guinea-pigs anaesthetized either with sodium barbitone or with urethane. Evidence will be presented indicating that, in the same experimental conditions, i.v. GABA can produce dual cardiovascular effects on blood pressure, heart rate and cardiac contractility dependent on factors such as (a) type of anaesthesia, (b) resting values of cardiovascular parameters, (c) presence of an intact sympathetic innervation and (d) catecholamine release from the adrenal medulla.

Methods

Male albino guinea-pigs (200–250 g) were anaesthetized either with i.p. sodium barbitone (350 mg kg^{-1}) or s.c. urethane (1.5 g kg^{-1}). These doses of the two anaesthetics were selected as being the minimal ones which allowed all surgical procedures to be performed without pain or discomfort for the animal. This was judged on the basis of absence of any withdrawal reaction upon incision of the skin. The animals were placed on a heating pad maintained at 37°C to keep the body temperature constant. The left carotid artery and the right jugular vein were cannulated with polyethylene tubing for blood pressure recording and drug administration, respectively.

Respiratory rate was measured by means of a MARB 76/01 impedance pneumograph connected to a Hewlett Packard (H.P.) 8802A medium gain amplifier. The tachypnoic response to anal stimulation was obtained by pinching the anal mucosa for 10 s with forceps. Skeletal muscle relaxation was evaluated by holding a fold of skin on the ventral surface of the thorax and looking for the presence of nuchal relaxation.

The blood pressure signal was recorded by means of an H.P. 1290A pressure transducer connected to a H.P. 88050 pressure amplifier which was used to trigger a H.P. 15050 cardiotachometer. Heart rate was recorded on a H.P. 8802A medium gain amplifier. Intraventricular pressure was determined as described by Hayes (1982) by inserting a 22 gauge needle through the thoracic wall in the left ventricle. The needle was inserted just to the left of the xiphoid process at an angle of 20° until the pressure tracing confirmed its entry into the left ventricle (Hayes, 1982). The needle was left free to follow the respiratory and/or cardiac movements. Heart rate, blood pressure, left ventricular pressure and its first derivative (dP/dt) were displayed on a four channel H.P. 7754A polygraph.

A three-way stopcock with a 2.5 ml syringe filled with heparinized (500 i.u ml^{-1}) saline was connected to the transducer to provide flushing of the needle, when necessary. Intracardiac pressure was recorded by means of a H.P.1290A pressure transducer connected to a H.P. 8805A carrier amplifier and a 8802A medium gain amplifier modified for the measurement of the first derivative (dP/dt), an accepted index of cardiac contractility (Mason, 1969).

Those preparations (about 5%) which, after an equilibration period of 15 min exhibited extrasystoles were discarded. Suitable preparations provided fairly steady recordings of intraventricular pressure for at least 2 h.

Some experiments were performed in bilaterally adrenalectomized or sham-operated guinea-pigs. Adrenalectomy was performed at least 60 min before GABA administration.

After completion of surgical procedures, cardiovascular parameters were allowed to equilibrate for at least 30 min. It was noted that reproducibility of GABA effects in the same animal varied with the type of anaesthesia (see the results section). However, to avoid tachyphylaxis only one dose of GABA was administered in each animal. Potential antagonists were injected intravenously 3–10 min before challenge with GABA. Some experiments were performed in reserpine pretreated animals (5 mg kg^{-1}, 24 h before induction of anaesthesia).

Statistical analysis

Each value in the text is mean ± s.e.mean. Statistical analysis of the data was performed by means of the Student's t test for paired or unpaired data, when applicable. Regression analysis was performed by means of the least squares method.

Drugs

Drugs used were: γ-aminobutyric acid (GABA, Serva), 3-amino-1-propanesulphonic acid (homotaurine, Aldrich), propranolol HCl (Sigma), reserpine (Serva), picrotoxin (Sigma), bicuculline methyl iodide (BMI, Sigma) (±)-baclofen (Ciba Geigy), muscimol (Serva), γ-hydroxybutyrate Na (Serva), hexamethonium bromide (Serva), phentolamine mesylate (Regitin, Ciba) urethane (ethyl carbamate (Serva), sodium barbitone (Merck), 4, 5, 6, 7- tetrahydroisoxazolo [5, 4- c] pyridin-3-ol (THIP) was a gift from H. Lundbeck & Co. (Copenhagen, Denmark). All drugs were dissolved and injected in 0.9% saline, in a volume of 1 ml kg^{-1}.

Results

There was no significant difference in values of systolic blood pressure (SBP, 82 ± 2 and 85 ± 2 mm Hg, $n = 40$ and 41) or diastolic blood pressure (DBP, 53 ± 1 and 47 ± 2 mm Hg) between barbitone- or urethane- anaesthetized guinea-pigs, respectively. On the other hand values of both heart rate (HR, 241 ± 5 and 293 ± 5 beats min^{-1}, respectively, $P < 0.01$) and dP/dt (3479 ± 186 and 6096 ± 200 mm Hg s^{-1}, respectively $P < 0.01$) were significantly lower in barbitone- as compared to urethane- anaesthetized animals.

The question was raised as to whether or not differences in the level of anaesthesia and/or anaesthetic-induced interference with ventilation may have influenced the effects of GABA in barbitone- as compared to urethane-anaesthetized animals. Both anaesthetics produced a similar degree of skeletal muscle relaxation. Respiratory rate was significantly lower in barbitone- than in urethane-anaesthetized

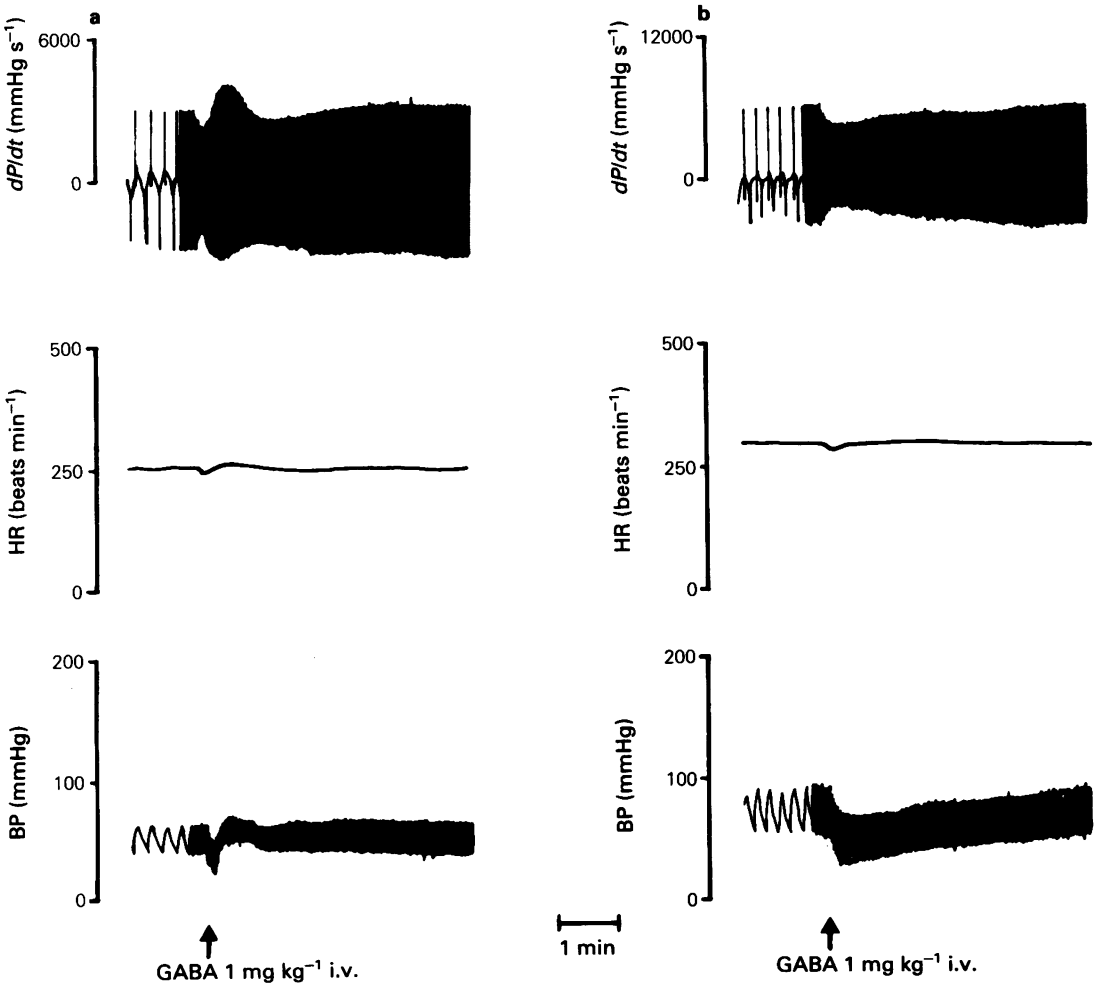

Figure 1 Typical tracings showing the cardiovascular effects (BP = blood pressure, HR = heart rate) of GABA (1 mg kg^{-1} i.v.) in barbitone- (a) or urethane- (b) anaesthetized guinea-pigs.

guinea-pigs (74 ± 5 and 105 ± 6 breaths min^{-1}, respectively, $n = 10$ for each group, $P < 0.05$). In barbiturate-anaesthetized animals there was no change in respiratory rate (75 ± 8 breath min^{-1}) following anal stimulation. On the other hand this manoeuvre produced a significant tachypnoeic response (125 ± 6 breaths min^{-1}, $P < 0.05$ as compared to controls) in urethane-anaesthetized guinea-pigs.

Effect of GABA on cardiovascular parameters in guinea-pigs anaesthetized with barbitone

GABA (0.01–10 mg kg^{-1}, i.v.) had little or no effect on heart rate of barbitone-anaesthetized guinea-pigs. A biphasic change in blood pressure and *dP/dt* was observed, (Figure 1) characterized by an initial decrease (10–20%) which reached its maximum within 15–30 s from GABA administration and followed by a more sustained (60–90 s) increase (about 15–20%) (Figures 1, 2 and 3) and return to resting values. With the possible exception of the initial transient decrease in blood pressure and *dP/dt*, the GABA-induced cardiovascular changes did not appear to be dose-related (Figure 2).

The amplitude of the initial reduction in SBP (Figure 4) and DBP ($r = 0.803$, $n = 9$, $P < 0.01$) produced by i.v. GABA (1 mg kg^{-1}) was significantly related to resting values. On the other hand, neither decrease in *dP/dt*, nor the delayed increase in SBP, DBP and *dP/dt* were significantly related to resting values (data not shown).

The 'delayed' positive inotropic effect of GABA (1–10 mg kg^{-1}) was not reproducible even if a 30 min interval was allowed to elapse between two doses. Although the effect of a lower dose of GABA (10–100 μg kg^{-1}) was somewhat more reproducible at

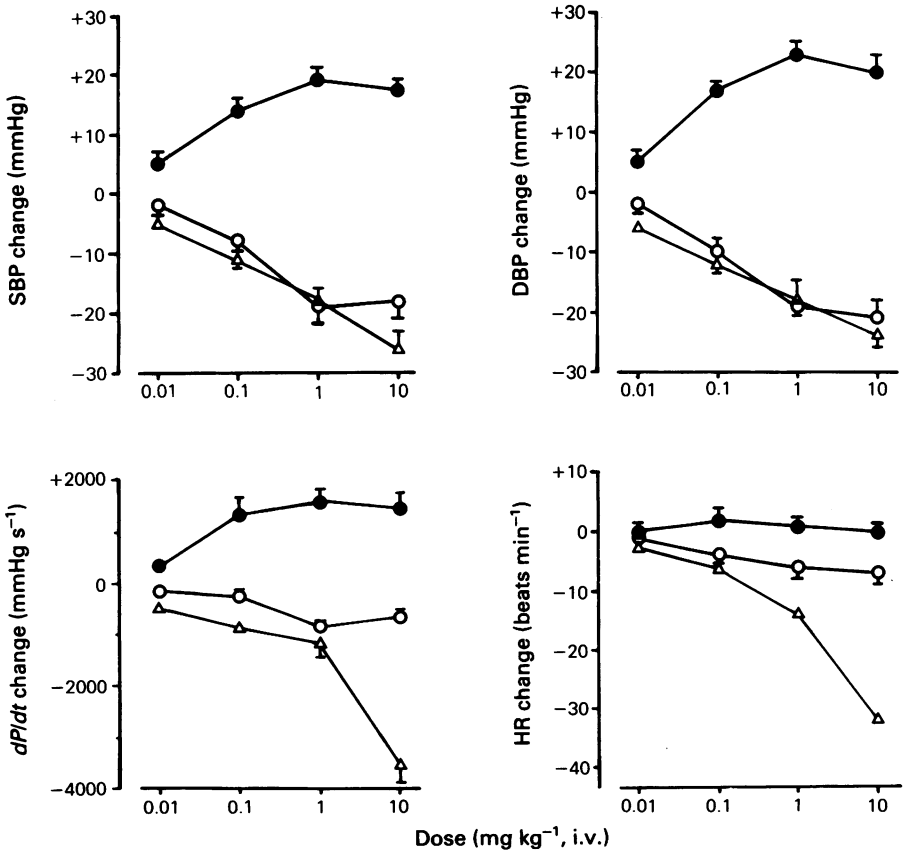

Figure 2 Dose-related cardiovascular effects, (SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate) of GABA in barbitone ('depressive' effects ○ 'excitatory' effects; ●) or urethane (△)-anaesthetized guinea-pigs. Each point is the mean of at least 6 experiments; s.e.mean shown by vertical lines.

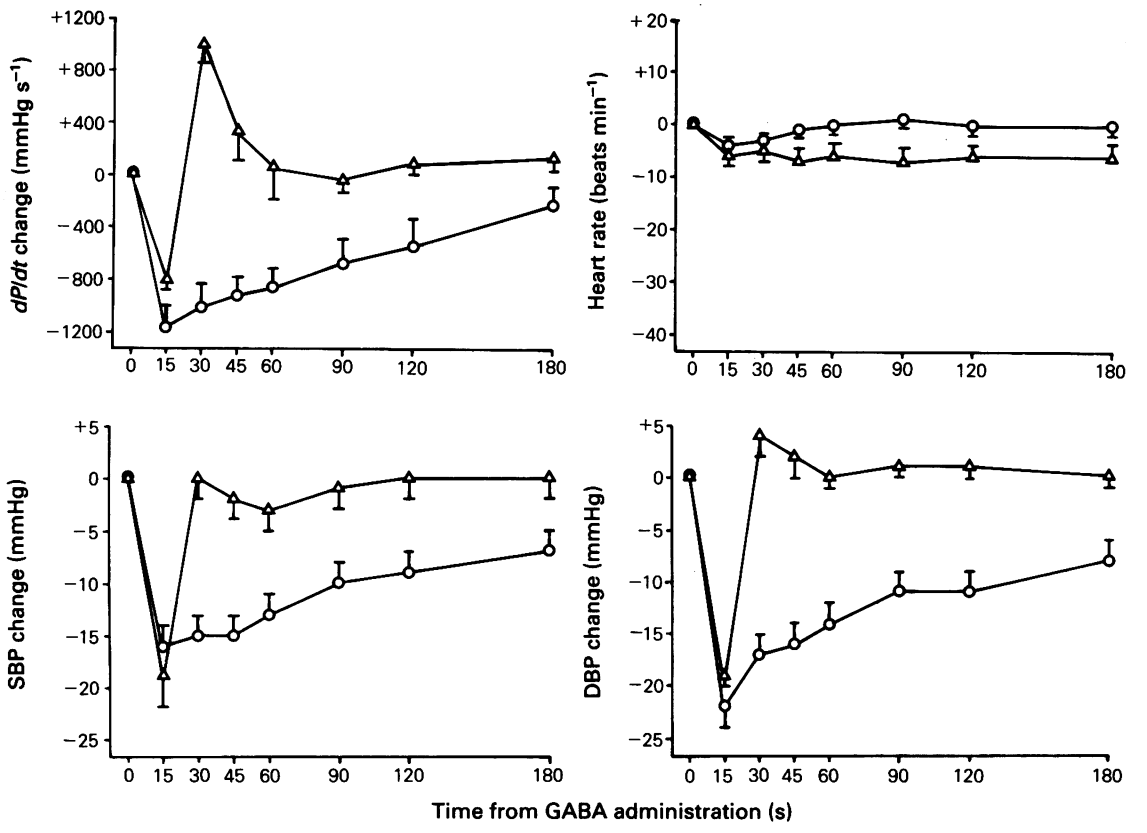

Figure 3 Time course of the cardiovascular effects (SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate) produced by i.v. GABA (1 mg kg^{-1}) in barbitone – (Δ) or urethane – (O) anaesthetized guinea-pigs. Each point is the mean of at least 6 experiments; s.e.mean shown by vertical lines.

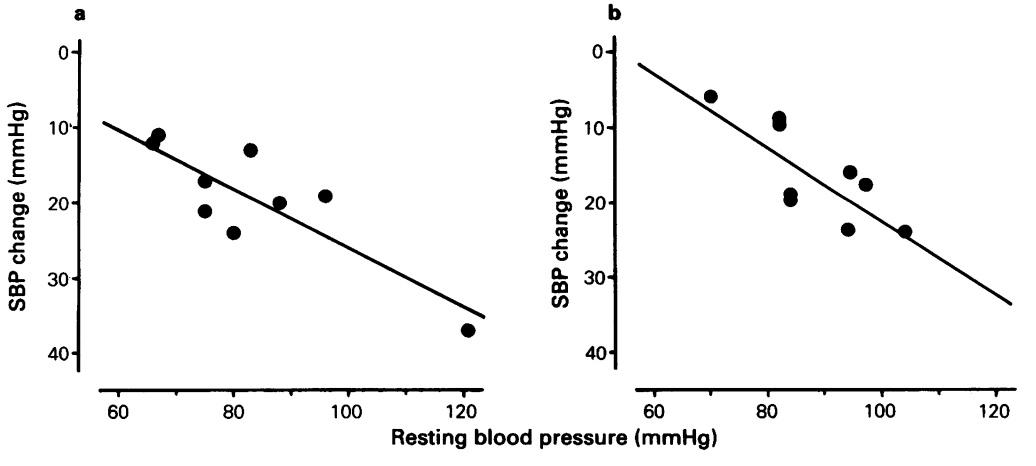

Figure 4 Relationship between resting blood pressure and amplitude of the GABA- (1 mg kg^{-1} i.v.) induced reduction in systolic blood pressure (SBP) in barbitone (a) or urethane (b) -anaesthetized guinea-pigs. Each point was obtained from a single animal; in (a) $n = 9$, $r = 0.8491$, $P < 0.01$; in (b) $n = 9$, $r = 0.7723$; $P < 0.01$.

15–30 min intervals, the effect of potential antagonists was investigated, to avoid desensitization, in preparation receiving only one dose of GABA.

Effect of GABA on cardiovascular parameters in guinea-pigs anaesthetized with urethane

GABA (0.01–10 mg kg^{-1} i.v.) produced a dose-related depression in heart rate, blood pressure and *dP/dt* of urethane-anaesthetized guinea-pigs (Figures 1, 2 and 3). All these changes appeared within a few seconds and their duration was dose-related.

The amplitude of GABA-induced (1 mg kg^{-1}) cardiovascular changes (SBP, DBP and *dP/dt*, Figure 3) is significantly related to resting values (DBP: $r = 0.7527$, $n = 9$, $P < 0.05$; *dP/dt*: $r = 0.7475$, $n = 9$, $P < 0.05$). The relationship between resting SBP values and amplitude of GABA- induced hypotension is shown in Figure 4. There was no significant relationship between the slight GABA-induced bradycardia and resting HR (data not shown).
The effects of GABA (0.01–10 mg kg^{-1} i.v.) on HR, *dP/dt* and blood pressure were reproducible providing that a 15–30 min interval elapsed between two GABA doses.

Effect of picrotoxin on GABA-induced cardiovascular changes in guinea-pigs anaesthetized with barbitone

Picrotoxin (2 mg kg^{-1}, i.v.) barely affected cardiovascular parameters of barbitone-anaesthetized guinea-pigs (Figure 5). As compared to controls, picrotoxin

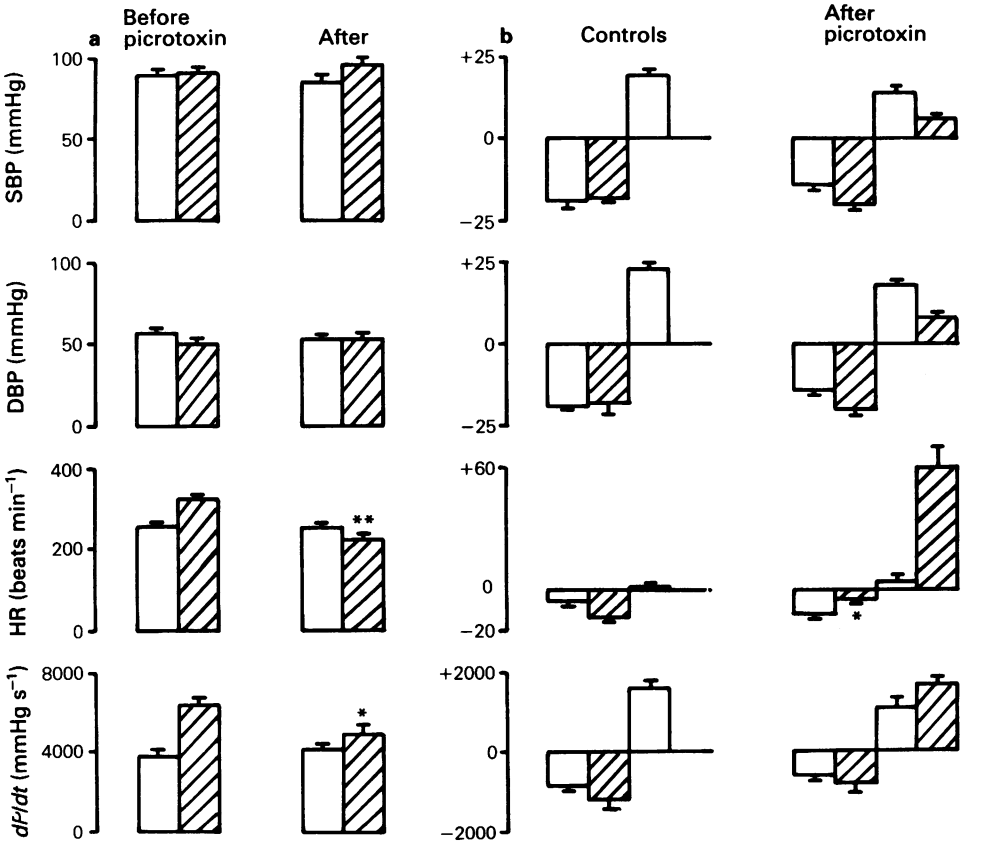

Figure 5 Effect of picrotoxin (1 mg kg^{-1} i.v.) on resting cardiovascular parameters (a) and on GABA- (1 mg kg^{-1}, i.v.) induced cardiovascular changes (b) in guinea-pigs anaesthetized with barbitone (open columns) or urethane (hatched columns). Note that in barbitone-anaesthetized guinea-pigs the effects of GABA were biphasic, i.e. an initial 'depressive' phase (hypotension, bradycardia etc.) was replaced by a delayed 'excitatory' phase (hypertension, tachycardia etc.). Under urethane anaesthesia only a depressive phase was observed in control animals but a delayed excitatory phase appeared after picrotoxin pretreatment. Each value is mean of at least 6 experiments; s.e.mean shown by vertical lines. Significantly different from controls, $^{*}P < 0.05$; $^{**}P < 0.01$.

pretreatment (10 min before) had no significant effect on the cardiovascular changes produced by i.v. GABA (1 mg kg^{-1}). Only the increase in *dP/dt* produced by i.v. GABA was lower in picrotoxin pretreated animals (about 30% inhibition) (Figure 5). This subconvulsant dose of picrotoxin antagonizes the GABA- (1 mg kg^{-1}, i.v.) induced enhancement of micturition contraction in anaesthetized guinea-pigs of the same age and strain (Maggi, unpublished data).

Effect of picrotoxin on GABA- induced cardiovascular changes in guinea-pigs anaesthetized with urethane

In urethane-anaesthetized guinea-pigs, picrotoxin (2 mg kg^{-1}, i.v.) barely affected blood pressure but induced a marked decrease in resting HR (about 30%) and *dP/dt* (about 25%, Figure 5). These effects ensued within 1–2 min, reached a steady state within 5–8 min and persisted for at least 10 min.

In picrotoxin pretreated (2 mg kg^{-1}, i.v. 10 min before) urethane- anaesthetized guinea pigs the effects of GABA (1 mg kg^{-1}) became biphasic and particularly, similar to those observed in barbitone-anaesthetized animals (Figure 5). Amplitude of initial inhibitory effect (Figure 5) was similar to that observed in controls (SBP -18 ± 2 mm Hg, DBP -18 ± 2 mm Hg, *dP/dt* -1171 ± 234 mm Hg s^{-1}, $n = 7$) with the exception of the GABA-induced bradycardia which was reduced by picrotoxin (-14 ± 1 and -5 ± 1 beats min^{-1} in controls and picrotoxin pretreated animals, respectively, $P < 0.01$). In picrotoxin pretreated animals the inhibitory phase was replaced, within a few seconds, by a delayed increase in cardiovascular parameters which had never been observed in controls (Figure 5).

Effect of bicuculline methyl-iodide (BMI) on GABA-induced cardiovascular changes in barbitone- or urethane-anaesthetized guinea-pigs

Since picrotoxin crosses the blood brain barrier (BBB) its usefulness as a tool to establish the involvement of peripheral mechanisms in the action of GABA is limited. BMI, a selective $GABA_A$ receptor antagonist which does not cross the BBB (Pong & Graham, 1972; Bowery *et al.*, 1981) was used to determine the potential involvement of peripheral $GABA_A$ receptors in GABA effects on the cardiovascular system. These experiments were carried out in urethane-anaesthetized guinea-pigs because in these conditions only the 'depressant' effects of GABA are present (see Figure 1). On the other hand the effects of BMI on GABA-induced increases in cardiovascular parameters were studied in conditions in which the 'depressant' component was absent (see below). BMI produced a transient (3–5 min) reduction (15–40%) of cardiovascular parameters.

Pretreatment (5 min before) with a dose of BMI as large as 5 mg kg^{-1} i.v did not affect the 'depressive' cardiovascular changes produced by i.v. GABA (1 mg kg^{-1}). This dose of BMI antagonizes the GABA- (1 mg kg^{-1}, i.v.) induced enhancement of micturition contraction in anaesthetized guinea-pigs (Maggi, unpublished data).

Effect of hexamethonium on GABA-induced cardiovascular changes in barbitone- or urethane-anaesthetized guinea-pigs

Regardless of initial values, hexamethonium (20 mg kg^{-1}, i.v.) induced a prompt and comparable decrease of SBP (about 60%), DBP (about 60%), HR (about 15–20%) and *dP/dt* (about 70%) in both barbitone- or urethane-anaesthetized guinea-pigs.

The cardiovascular changes induced by hexamethonium lasted for at least 20 min and during this period the effects of i.v. DMPP (0.1 mg kg^{-1}) (increases in SBP, DBP, RHR and *dP/dt*) were almost suppressed ($n = 5$, data not shown).

In hexamethonium pretreated animals (20 mg kg^{-1} i.v., 5 min before) the cardiovascular effects produced by i.v. GABA were abolished or markedly (over 80%) reduced, irrespectively of the anaesthetic used (Figure 6).

Effect of reserpine pretreatment on GABA-induced cardiovascular changes in barbitone- or urethane-anaesthetized guinea-pigs

Reserpine pretreatment (5 mg kg^{-1} i.p. 24 h before) markedly lowered resting cardiovascular parameters in both barbitone- and urethane-anaesthetized animals. In fact SBP (52 ± 3 and 57 ± 2 mm Hg, $n = 6$ and 8, respectively) DBP (33 ± 2 and 31 ± 2 mm Hg, respectively) HR (165 ± 10 and 224 ± 10 beats min^{-1}, respectively) and *dP/dt* (1867 ± 169 and 3040 ± 21211 mm Hg s^{-1}, respectively) were significantly lower in both barbitone- or urethane-anaesthetized than in their own control group.

Chemical sympathectomy at cardiovascular level was assessed by determining the effects of i.v. tyramine (0.1 mg kg^{-1}). In control animals i.v. tyramine induced a marked increase in *dP/dt* (3400 ± 250 mm Hg s^{-1}, $n = 5$) which was significantly reduced (over 85% inhibition) by reserpine pretreatment.

The cardiovascular changes produced by GABA (1 mg kg^{-1}) in reserpine-pretreated barbitone-anaesthetized guinea-pigs were qualitatively different from those in controls. In fact, in reserpine pretreated animals GABA induced a slight tachycardia (8 ± 2 beats min^{-1}, $n = 6$) and increased both SBP and DBP (21 ± 3 and 17 ± 2 mm Hg, respectively). On the other hand the GABA-induced positive inotropic effect observed in controls was reduced by about 30% by

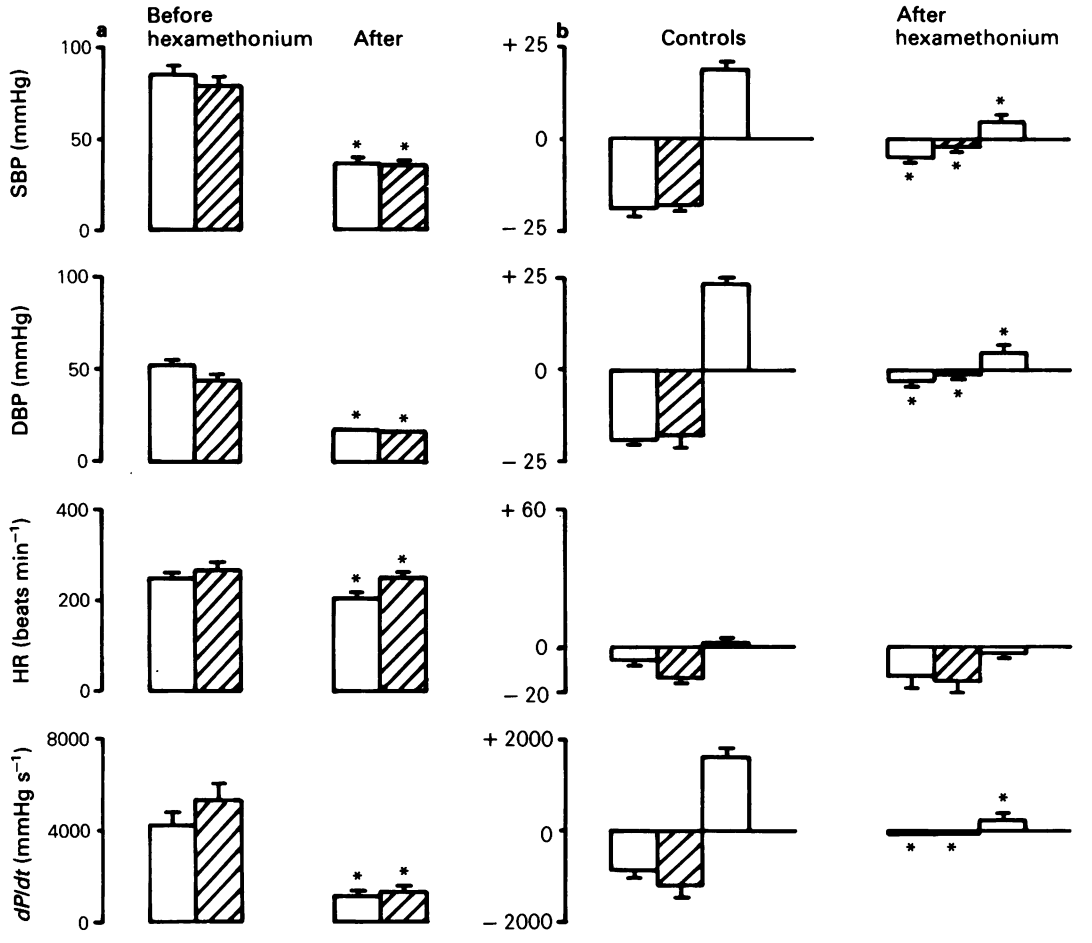

Figure 6 Effect of hexamethonium (20 mg kg^{-1}, i.v.) on resting cardiovascular parameters (a) and on GABA- (1 mg kg^{-1}, i.v.) induced cardiovascular changes (b) in guinea-pigs anaesthetized with barbitone (open columns) or urethane (hatched columns). Each value is mean of at least 6 experiments, s.e.mean shown by vertical lines. Significantly different from controls, $P<0.01$.

reserpine pretreatment. The inhibitory phase which, in normal animals, preceded the delayed excitatory component of the effect of GABA was almost abolished by reserpine pretreatment. Reserpine pretreatment prolonged up to 3–5 min the duration of GABA-induced positive inotropic effect.

Reserpine pretreatment also altered markedly the effect of GABA in urethane-anaesthetized animals. In fact a pressor (15 ± 2 and 13 ± 2 mm Hg for SBP and DBP, respectively) instead of a depressor episode and a positive (1480 ± 159 mm Hg s^{-1}) instead of negative inotropic effect followed GABA administration. On the other hand the bradycardia induced by GABA was unaffected by reserpine pretreatment.

Effect of GABA receptor agonists on cardiovascular parameters in reserpine pretreated, barbitone-anaesthetized guinea-pigs

These experiments were performed with the aim of characterizing the GABA receptor subtype involved in determining the pressor, tachycardic and positive

inotropic effect of i.v. GABA. Barbitone-anaesthetized reserpine pretreated guinea-pigs were used because, in these experimental conditions the excitatory effect of GABA on various cardiovascular parameters was not preceded by an inhibitory component.

$GABA_A$ receptor agonists, homotaurine (1.35 mg kg^{-1}, $n = 4$), THIP (1 mg kg^{-1}, $n = 4$) muscimol (1 mg kg^{-1}, $n = 4$) and 5-aminovaleric acid (1 mg kg^{-1}, $n = 4$) produced an effect on cardiovascular parameters similar to that produced by GABA (1 mg kg^{-1}) while γ-hydroxybutyrate had no effect even at a dose of 10 mg kg^{-1} ($n = 4$).

On the other hand (±)-baclofen (2 mg kg^{-1}, $n = 6$), a selective $GABA_B$ receptor agonist (Bowery *et al.*, 1981) produced hypotension and negative chronotropic effect (about 20% reduction of SBP and *dP/dt*, respectively). GABA (1 mg kg^{-1}) administered within 5 min of (±)-baclofen produced an increase in SBP, DBP, *dP/dt* and HR similar to that observed in controls. In contrast, administration of GABA within 5 min of muscimol (1 mg kg^{-1}, i.v.) administration had no 'excitatory' cardiovascular effects. Likewise a second administration of GABA within 5 min of the first dose did not affect cardiovascular parameters. Previous administration of DMPP (0.1 mg kg^{-1}, 5 min before) which produced excitatory cardiovascular effects did not affect the excitatory cardiovascular changes produced by i.v. GABA.

Effect of BMI or picrotoxin on GABA-induced cardiovascular changes in reserpine pretreated barbitone- anaesthetized guinea-pigs

Both BMI (5 mg kg^{-1}, i.v., $n = 6$) or picrotoxin (2 mg kg^{-1}, i.v., $n = 6$) barely affected resting cardiovascular parameters (data not shown) and did not modify the cardiovascular changes produced by a subsequent injection of GABA (1 mg kg^{-1}) (Figure 7) except that the increase in SBP and the positive inotropic effect of the latter were potentiated in picrotoxin-treated animals (Figure 7).

Effect of GABA on cardiovascular parameters in adrenalectomized barbitone-anaesthetized guinea-pigs

GABA (1 mg kg^{-1}, i.v.) effects in sham-operated animals were similar to those observed in controls. On the other hand the only GABA effect in adrenalectomized guinea-pigs was a reduction of cardiovascular parameters, i.e. the 'delayed' excitatory phase was abolished (data not shown). To characterize further this phenomenon we studied the effect of GABA in sham-operated and adrenalectomized guinea-pigs after reserpine pretreatment (Figure 7). In adrenalectomized preparations ($n = 6$) GABA produced only a slight inhibition of cardiovascular parameters. Sham-operated animals did not differ from controls (Figure 7).

Effect of phentolamine plus propranolol on GABA-induced cardiovascular changes in reserpine pretreated barbitone-anaesthetized guinea-pigs

Phentolamine (0.2 mg kg^{-1}) plus propranolol (1 mg kg^{-1}) reduced *dP/dt* (about 50%) HR (about 20%) and blood pressure (5–10%) (data not shown). This dose of phentolamine plus propranolol antagonized the noradrenaline-induced relaxations of the distal colon in anaesthetized guinea-pigs (Maggi *et al.*, 1985). In these animals GABA (1 mg kg^{-1}, 5–10 min later) produced only a slight further reduction of cardiovascular parameters ($n = 6$, Figure 7).

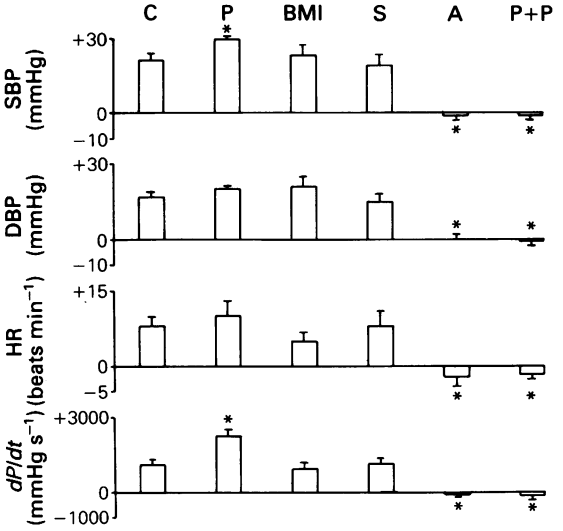

Figure 7 Effect of GABA (1 mg kg^{-1}, i.v.) on cardiovascular parameters (SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate) in reserpine pretreated (5 mg kg^{-1}, i.p., 24 h before) barbitone-anaesthetized guinea-pigs. C = control group, P = picrotoxin pretreated (2 mg kg^{-1}, i.v.), BMI = bicuculline methyliodide (5 mg kg^{-1}, i.v.), S = sham-operated animals (controls for adrenalectomy group), A = bilateral adrenalectomy, P + P = phentolamine (0.2 mg kg^{-1}, i.v.) plus propranolol (1 mg kg^{-1}, i.v.). Each value is mean of at least 6 experiments; s.e.mean shown by vertical lines. Significantly different from controls, $*P < 0.01$.

Discussion

The cardiovascular changes produced by i.v. GABA are most probably due to its effect(s) at a peripheral site (cf. Maggi *et al.* 1983; 1985a; 1986). On the other hand it appears unlikely that the GABA-induced cardiovascular changes are due to a direct effect on the heart (Elliott & Hobbiger, 1959) or blood vessels, since they were abolished by hexamethonium pretreatment. This suggests that the action of GABA is due to interference with the function of the autonomic nervous system indicating a 'neuromodulatory' effect of GABA on cardiovascular function as already described for the guinea-pig isolated ileum (Giotti *et al.*, 1983) and urinary bladder (Maggi *et al.* 1985c,d). In fact, changes of opposite direction in cardiovascular parameters were observed depending upon resting conditions and/or activation of different GABA receptor subtypes (see below).

Our findings demonstrate that GABA produces two opposite sets of cardiovascular changes i.e. 'depressive' (hypotension, bradycardia and negative inotropic effect) or 'excitatory' (hypertension, tachycardia, positive inotropic effect) which are qualitatively and quantitatively dependent upon the influence of the anaesthetic on resting cardiovascular function.

Urethane is known to produce a condition of surgical anaesthesia characterized by a high resting 'tone' of the autonomic nervous system in controlling cardiovascular function (Maggi & Meli, 1986). On the other hand, barbiturates have some ganglion blocking properties (Larrabee & Posternak, 1954). This may account for the lower values in some cardiovascular parameters observed in barbitone- as compared to urethane-anaesthetized animals. Accordingly, the 'excitatory' effects of GABA may be seen only when the sympathetic cardiovascular 'tone' is low. In fact, in urethane-anaesthetized animals, GABA induced an 'exctiatory' effect following reserpine pretreatment.

Another factor which may be involved in determining the anaesthesia-related cardiovascular response to GABA is the very different resting respiratory rate, which was almost normal in animals anaesthetized with urethane (cf. Advenier *et al.*, 1978) but markedly depressed under barbitone anaesthesia. However, experiments in artificially ventilated, barbitone- or urethane-anaesthetized guinea-pigs indicated that the cardiovascular changes produced by i.v. GABA ($1\ mg\ kg^{-1}$) are similar both qualitatively and quantitatively to those observed in animals breathing spontaneously (Giuliani, unpublished data).

The 'depressive' effects of GABA on cardiovascular function have been described in a number of studies (Takahashi *et al.*, 1955; 1959; Elliott & Hobbiger, 1959; Stanton, 1963; Vemulapalli & Barletta, 1984; Maggi *et al.*, 1985a) and may be ascribed to: (a) interference with transmission in sympathetic ganglia (Kato & Kuba, 1980; Kushiku & Furukawa, 1985; Maggi *et al.*, 1985c,d) and/or (b) a prejunctional inhibition of transmitter release from sympathetic nerve endings (Starke & Weitzell, 1980; Bowery *et al.*, 1981; Anwar & Mason, 1982; Muhyaddin *et al.*, 1983; Manzini *et al.*, 1985). It is quite possible that the 'depressive' effects of GABA observed in the present study depend upon a transient blunting of sympathetic control on cardiovascular function since the amplitude of 'depressive' changes induced by GABA was proportional to resting values of cardiovascular parameters.

The 'excitatory' effects of i.v. GABA on cardiovascular function has previously been described, to our knowledge, only twice, and the mechanisms involved were not determined (Stanton & Woodhouse, 1960; Horvath *et al.*, 1980). Our findings indicate that the pressor effect of GABA could be mainly mediated through a release of catecholamines from the adrenal medulla. In fact the 'excitatory' cardiovascular effects of GABA were prevented by previous administration of phentolamine plus propranolol and were not observed in adrenalectomized animals.

Recent data demonstrate that GABA possesses a 'neuromodulatory' action on chromaffin cells of the adrenal medulla in various species since both excitatory and inhibitory effects have been demonstrated (Sangiah *et al.*, 1974; Kitayama *et al.*, 1984; Kataoka, *et al.*, 1984).

In our experimental conditions the 'excitatory' cardiovascular changes produced by i.v. GABA were prevented by hexamethonium. This suggests that GABA has an excitatory effect on some peripherally located cholinergic neurone innervating the adrenal medulla which mediates catecholamine release through activation of a nicotinic receptor.

To date, GABA receptors in the peripheral nervous system appear to be similar in term of agonist and antagonist selectivity, to GABA receptors in the CNS (Bowery *et al.*, 1983). Bowery *et al.* (1981) described two major subtypes of GABA receptors ($GABA_A$ and $GABA_B$) whose pharmacological identification relies on three main criteria: (a) agonist selectivity in producing a given biological effect; (b) cross-desensitization between GABA and selective $GABA_A$ (muscimol, homotaurine) or $GABA_B$ (baclofen) receptor agonists and (c) antagonism by selective $GABA_A$ (bicuculline, picrotoxin) or $GABA_B$ receptor antagonists (5-aminovaleric acid, homotaurine).

The 'depressive' cardiovascular changes produced by i.v. GABA in anaesthetized guinea-pigs may involve activation of peripheral $GABA_B$ receptors since they were substantially bicuculline-insensitive. Peripheral $GABA_B$ receptors were shown to be activated by GABA in urethane- anaesthetized guinea-pigs and modulate colonic motility (Giotti *et al.*, 1985) at doses similar to those used in the present study and may thus

be involved in the cardiovascular action of GABA.

The GABA-induced 'excitatory' cardiovascular changes seem to be mediated by a '$GABA_A$- like' receptor subtype since: (a) they are mimicked by a series of $GABA_A$ receptor agonists; (b) cross desensitization was observed between muscimol and GABA and (c) (±)-baclofen, a selective $GABA_B$ receptor agonist, did not mimic nor depress the effects of GABA. On the other hand, the 'excitatory' cardiovascular effects of GABA were not prevented even by large doses of conventional $GABA_A$ receptor antagonists such as BMI or picrotoxin. These latter findings are at variance with previous observations indicating that $GABA_A$ receptor antagonists prevent the GABA-induced catecholamine release from the bovine (Sangiah *et al.*, 1974) and canine (Kitayama *et al.*, 1984) adrenal medulla. It appears therefore that 'atypical' $GABA_A$ receptors may be involved in producing catecholamine release from the guinea-pig adrenal medulla.

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