ELSEVIER

Behavioural Brain Research 62 (1994) 11–20

BEHAVIOURAL BRAIN RESEARCH

Research Report

Cardiovascular effects of the benzodiazepine receptor partial inverse agonist FG 7142 in rats

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Received 25 May 1993; revised 17 January 1994; accepted 17 January 1994

Abstract

Effects of the benzodiazepine receptor (BZR) partial inverse agonist FG 7142 (FG) on basal and reactive cardiovascular measures were examined in freely moving rats. FG (8 mg/kg) modestly increased basal heart period, but had no effects on basal blood pressure. More notably, however, FG augmented the cardioacceleratory response to an auditory stimulus relative to vehicle controls. Selective blockade of sympathetic (atenolol, 1 mg/kg) or parasympathetic (scopolamine methylnitrate, 0.1 mg/kg) effects on the heart under control conditions revealed that the stimulus-evoked cardiac response originated from a concurrent (reciprocal) sympathetic activation and vagal withdrawal. Following FG pretreatment, both atenolol and scopolamine blocked the cardioacceleratory response to the auditory stimulus. Thus, although FG minimally increased basal heart period, FG significantly enhanced a reactive cardioacceleration. More importantly, these results demonstrate that the cardiovascular effects of BZR inverse agonists are more fully characterized by an assessment of both tonic and reactive cardiovascular responses.

Key words: Autonomic; Sympathetic; Parasympathetic; Cardiac reactivity; Blood pressure reactivity

1. Introduction

FG 7142 (*N'*-methyl-β-carboline-3-carboxamide) has been classified as a benzodiazepine receptor (BZR) partial inverse agonist on the basis of its ability to inhibit GABAergic transmission [9], to exert proconvulsant effects at relatively high doses [25] and to exert proconflict effects [38,39]. However, in contrast to BZR full inverse agonists, FG 7142 does not produce seizures when given acutely.

The proconflict effects of FG 7142 shown in earlier animal experiments have been assumed to reflect drug-induced stress or anxiety. This hypothesis has become a subject of intense debate since the report on the effects of this drug in human volunteers [18]. Although the findings from this open, uncontrolled trial may not represent a valid basis for conclusions about the anxiogenic properties of FG 7142 (see ref. 51), subsequent animal studies have supported the hypothesis that this drug produces behavioral effects which mimic anxiety. This provides some face validity for the view that FG 7142 is anxiogenic (e.g. refs. 2, 3, 40, 50).

Several studies have suggested the possibility that the anxiogenic or stressor-like effects of FG 7142 may be mediated via a selective increase in metabolic activation of the dopaminergic prefrontal cortical input, a result consistent with the effects of environmental stressors [26,31]. In addition, GABAergic systems are modulated directly by BZR ligands, and in turn, several cholinergic systems are modulated by GABAergic input. Both neurotransmitter systems have been implicated in the response to stress [11,16,21,43,53].

Although BZR partial inverse agonists appear to exert anxiogenic behavioral effects, their potential anxiety-related cardiovascular actions have not been fully characterized. Administration of the BZR inverse agonist, β-CCE (ethyl-β-carboline-3-carboxylate) in animals has been reported to elevate heart rate and blood pressure, as well as to increase plasma cortisol and catecholamines, consistent with the putative anxiogenic actions of this compound [13,35,45]. Little data exist, however, on the cardiovascular effects of FG 7142. An ex vivo study of the isolated rat heart revealed that FG 7142 has no direct effects on

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SSDI 0166-4328(94)00012-5

either the inotropic or chronotropic state of the heart, nor does it alter the cardiac response to norepinephrine [46]. Dorow et al. [18] reported that, in one human subject, administration of 200 mg FG 7142 resulted in an increase in blood pressure (from 105/50 to 160/100 mmHg) and pulse rate (from 80 to 110 bpm). These effects were accompanied by reports of severe anxiety, tension and agitation. Although these initial reports are intriguing, the potential cardiovascular actions of FG 7142 have yet to be fully explored.

A comprehensive characterization of the potential cardiovascular effects of FG 7142 may require more than a simple cataloging of effects on cardiovascular end-organ measures. The mere identification of an effect on heart period or blood pressure does not provide a comprehensive picture of the underlying autonomic bases of these adjustments [4]. Iwata and LeDoux [24] demonstrated that although different behavioral challenges may yield similar cardioacceleratory responses, selective pharmacological blockades of the autonomic branches may reveal fundamental differences in the autonomic origins of these responses. Whereas the cardioacceleratory response of a pseudoconditioned control group arose from a pattern of reciprocal sympathetic activation and parasympathetic withdrawal, the response of a conditioned group appeared to reflect a coactivation of both autonomic divisions. Thus, the functional output of a dually-innervated target organ such as the heart may be equivocal with respect to its underlying autonomic origins.

The present experiment investigated the effects of the BZR partial inverse agonist FG 7142 on basal cardiovascular state and reactive response to a nonsignal auditory stimulus. In addition, the autonomic origins of the cardiovascular effects were assessed by selective pharmacological blockade of the independent sympathetic and parasympathetic contributions.

2. Material and methods

2.1. Subjects

Subjects were 12 male Sprague–Dawley rats (Zivic Miller, Zelienople, PA) weighing 475 ± 71 g (mean ± S.D.) at the beginning of the experiment. Subjects were maintained on a 12 h light/dark cycle with testing during the light cycle. Subjects had ad libitum access to food and water.

2.2. Surgical procedure

Subjects were instrumented under anesthesia with a chronic catheter implanted into the right common carotid artery for measurement of heart period (inverse of heart rate) and blood pressure. The silastic catheter was attached to a septum-covered reservoir with an affixed collar that was exteriorized at the neck (Vascular Access Port, Access Technologies, Skokie, IL). Following anesthesia with ketamine and xylazine (90 mg/kg and 6 mg/kg, i.p., respectively), the animal was shaved and incisions made in the ventral and dorsal cervical areas. The right vagus and other nerve fibers were carefully dissected from the right carotid artery and the artery was ligated rostrally. An incision was made in the arterial wall and the catheter (0.51 mm i.d. × 0.84 mm o.d.) was advanced into the aortic arch and secured. Heart period measurements made from pressure pulses at the heart do not include the delays inherent in pulse frequency measures made at more distal sites. The vascular access port was exteriorized at the dorsum. Heparinized saline (10 USP units/cc) was administered via the catheter during surgery and 2–4 times per day thereafter (0.1–0.2 ml bolus). Subjects were administered dextrose and penicillin (15 mg in 0.3 cc and 60,000 units in 0.2 cc, respectively) immediately following surgery. Animals were allowed 24 h recovery before testing. This regimen maximized the number of subjects whose catheters remained patent for all 4 test days.

2.3. Drugs

The dose of the benzodiazepine receptor inverse agonist FG 7142 (8 mg/kg, i.p., suspended in a volume of 10% cremofor EL (BASF, Ludwigshafen, Germany) equivalent to 0.1% body mass) was chosen on the basis of a pilot dose response study and from data in the literature, to yield measurable cardiovascular actions, while minimizing the likelihood of proconvulsant activity. Pilot dose–response data revealed an FG 7142-induced enhancement of the reactive cardioacceleratory response only at the highest dose of FG 7142 (Doses and integral areas under the response ± S.E.M.: 0 mg/kg = 50.5 ± 21.8 ms, 2 mg/kg = 39.5 ± 17.5 ms, 4 mg/kg = 37.0 ± 13.0 ms, and 8 mg/kg = 115.6 ± 44.8 ms). Doses larger than 8 mg/kg were not used due to the potential pro-convulsive effects of FG 7142 with repeated administration, and the necessity for such re-administration in the present protocol. Although previous studies demonstrated that electrocortical records evidenced epileptogenic activity at 30 mg/kg, electrocortical patterns were unaffected by more moderate doses of FG 7142 (3 or 10 mg/kg; 48). The dosage employed is within the reported range for the induction of putative anxiogenesis [38,39]. Finally, pilot doses of FG 7142 did not yield noticeable effects on the general behavior of the subjects.

Autonomic antagonists were chosen to maximize peripheral action and to provide relatively complete

blockade of the autonomic control of the heart [41]. Scopolamine methylnitrate (0.1 mg/kg, s.c., in a volume of 0.1 mg/ml) was employed to block parasympathetic control of the heart. The quaternary compound was used to minimize central effects as it is slower to cross the blood brain barrier, especially at moderate doses [23,32]. Atenolol, (1 mg/kg, s.c., in a volume of 1.0 mg/ml), a relatively "cardio-selective" β_1 antagonist was administered for blockade of sympathetic effects on the heart [14,20,30]. Dose–response functions illustrating the effects of each of these agents on cardiac responses to nonsignal stimuli such as those used here are depicted in Quigley and Berntson [41].

Physiological recording apparatus and stimulus presentation. Blood pressure was recorded via a pressure transducer (Model TNF-R, Columbus Instruments, Columbus, OH) which was coupled to a polygraph (Model 7, Grass Instruments, Quincy, MA) for signal amplification and filtration (DC to 15 Hz). The analog signal was then passed to a computer for A/D conversion (500 Hz, 12 bit) and storage of the digitized data for offline processing.

Auditory challenge stimuli were presented through a free-field speaker located above the subject. Square-wave stimuli (1000 Hz, 20 s duration) were presented at 60 db (SPL) by a signal generator (BK Precision, Chicago, IL). A background white noise stimulus (50 db SPL) was presented continuously.

2.4. Experimental design and procedure

Each subject received FG 7142 (or its vehicle) followed by an autonomic antagonist (or its vehicle) in a 2×2 design. Subjects were divided into two groups of six, matched for reactivity to the auditory stimulus under vehicle conditions, with each group receiving one of the two autonomic blockers. Each subject received the following pretreatment/treatment regimen in block-randomized order over a 4 day period: (1) pretreatment vehicle, autonomic antagonist vehicle; (2) pretreatment vehicle, autonomic antagonist; (3) FG 7142, vehicle; (4) FG 7142, autonomic antagonist. For testing, subjects were placed inside a glass enclosure (51 cm × 30 cm × 25 cm) in a sound-attenuated, electrically shielded recording chamber (Industrial Acoustics, Inc., Bronx, NY). Cardiovascular measures were recorded over discrete 30 s trials. A 10-min adaptation period began each session immediately followed by three 30-s baseline trials. FG 7142 or its vehicle was administered immediately following the third baseline trial, and 20 min later, the autonomic antagonist or its vehicle was administered. Ten min after this second injection, 3 drug baseline trials were recorded, followed by 6 presentations of the auditory stimulus at a variable 2 min ITI.

2.5. Data reduction and analysis

Data were examined both visually and with a computerized algorithm for identification and correction of movement or recording artifacts [8]. Trials in which the prestimulus baseline was unstable, or in which excess movement artifacts were apparent were removed from the analysis. This resulted in removal of less than 8% of the data.

Analysis of variance was used to examine the effects of FG 7142 and autonomic blockade on basal heart period (ms) and blood pressure (mmHg). Mean basal heart period and blood pressure values were derived from the three, 30-s baseline trials. Cardiovascular responses to the auditory stimulus were quantified by deriving the integral area (in ms) under the pre- and poststimulus heart period and blood pressure response curves for three, 9-s time blocks. Because trials did not necessarily begin coincident with the onset of a heart period, a 27-s period from each trial was used for analysis following removal of the first 1 and last 2 s of the 30-s trial. This resulted in 1 prestimulus and 2 poststimulus time blocks. The integral data were submitted to repeated measures ANOVAs to examine the effects of FG 7142 on reactive heart period and blood pressure. Because separate analyses were conducted for pressor and depressor components of the blood pressure response, the P value for these analyses was adjusted to 0.025. Post-hoc comparisons were made using Fisher's least significant difference method.

Autonomic blockades. Autonomic blockades provide a means of estimating the independent contributions of the autonomic branches to an observed, unblocked response [6]. A given blockade, in fact, provides an estimate of both autonomic branches. The change in response after blockade of a single branch reflects the *subtractive* loss of that branch, and thus, the difference between the observed responses in the unblocked and blocked conditions offers an estimate of the normal contributions of the blocked branch. In contrast, any remaining response after blockade (assuming complete antagonism) indexes the *residual* contribution of the unblocked branch. Selective blockades of both autonomic divisions thus provide two separate estimates of the contributions of each branch. One of these estimates is derived by the subtractive method from blockade of the target branch, and the second is derived by the residual method from blockade of the other autonomic division.

Because the two estimates of a given branch are derived from distinct blockade conditions, they are differentially sensitive to potential systematic biases that can arise from pharmacological blockade. As is enumerated elsewhere [6], these biases can arise from both physiological (e.g. interactions between the branches at the level of the organ,

indirect reflexive alterations in the unblocked branch) or methodological factors (e.g. non-selective drug actions at remote sites, incomplete blockades). We have shown formally that any bias related to a given blockade condition will inversely affect the residual and subtractive model estimates of the two autonomic branches [6]. Systematic biases in blockade studies manifest in discrepancies between the two (residual and subtractive) estimates of a given autonomic branch. These discrepancies, therefore, index the biases in blockade data and offer a validity metric for interpretations of autonomic blockades.

3. Results

3.1. Effects of FG 7142 on basal heart period and blood pressure

FG 7142 resulted in a moderate increase in basal heart period (decreased heart rate), but did not alter basal blood pressure as evidenced by the results of Time (Pre-Drug/Post-Drug) × Drug (Vehicle/FG 7142) ANOVAs on mean basal heart period (ms) and blood pressure (mmHg). The FG 7142-induced increase in heart period was indicated by a significant Time × Drug interaction. Post hoc tests revealed that heart period was significantly increased following FG 7142 (Interaction: $F_{1,11} = 6.29$, $P < 0.05$; mean post-vehicle heart period: 175.6 ± 4.3 ms; mean post-FG 7142 heart period: 192.8 ± 6.0 ms; Post-vehicle vs. post-FG 7142 comparison: $P < 0.05$). Conversely, FG 7142 did not alter basal blood pressure as evidenced by a lack of main effects or interactions (Drug main effect and interaction: $Fs_{1,11} < 1.0$, $Ps > 0.3$; mean post-vehicle blood pressure: 111.1 ± 2.8 mmHg; mean post-FG blood pressure: 108.0 ± 2.3 mmHg).

3.2. Effects of FG 7142 on phasic heart period and blood pressure responses

Reactive heart period and blood pressure responses to the auditory stimulus were submitted to separate Time Block (Pre-stimulus/Post-stimulus 1/Post-stimulus 2) × Drug (Vehicle/FG 7142) repeated measures ANOVAs. Because virtually all cardiac responses were accelerator, analyses were based on integral area measures (in ms) under the cardioacceleratory heart period response function[1]. A significant acceleratory response to the stimulus was revealed by a significant main effect of Time Block ($F_{2,22} = 162.07$, $P < 0.001$). Post-hoc analyses revealed that reactive heart period responses following both vehicle and FG 7142 pretreatment were significantly different from baseline (all Prestimulus vs. Poststimulus 1 or 2 comparisons significant; $P < 0.01$). Furthermore, the response to the challenge stimulus after both FG 7142 and vehicle pretreatment was relatively stable over the two poststimulus time blocks (Poststimulus 1 vs. Poststimulus 2: $P > 0.05$). Reactive heart periods following FG 7142 were enhanced relative to vehicle controls as revealed by a significant main effect of Drug ($F_{1,11} = 16.13$, $P = 0.002$; Fig. 1). These results demonstrate that the tachycardic response to an auditory stimulus is significantly enhanced by FG 7142.

Subjects exhibited significant pressor and depressor responses following the auditory stimulus (pressor and depressor Time Block main effect $Ps < 0.001$; Fig. 1). Although pretreatment with FG 7142 did not alter the pressor response to the auditory stimulus, the stimulus-evoked depressor response was significantly larger after FG 7142

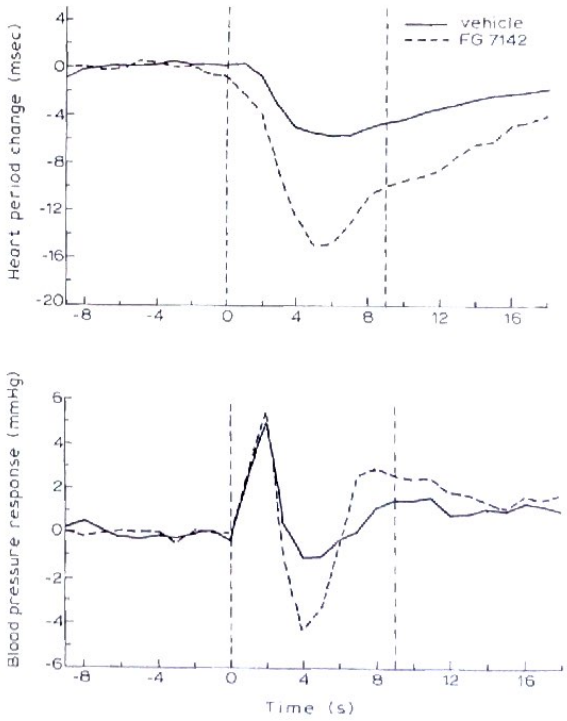

Fig. 1. The effects of vehicle and FG 7142 pretreatment on reactive heart period (ms) and blood pressure (mmHg) are depicted. Mean data ($n = 12$) are presented as changes from prestimulus baseline values. The dashed vertical lines delineate the 3 analysis periods: prestimulus, and poststimulus 1 and 2. The dashed vertical line at time = 0 also indicates stimulus onset. Data illustrate that FG 7142 pretreatment heart period response was enhanced relative to vehicle pretreatment and the transient depressor response following FG 7142 was enhanced relative to vehicle controls.

[1] Because the assumption of homogeneity of variance was violated by the reactive heart period integral areas, analyses were performed on log-transformed integrals. Ancillary analyses on untransformed heart period integrals revealed results comparable to those obtained with log-transformed heart periods for the effects of both FG 7142 and autonomic blockade.

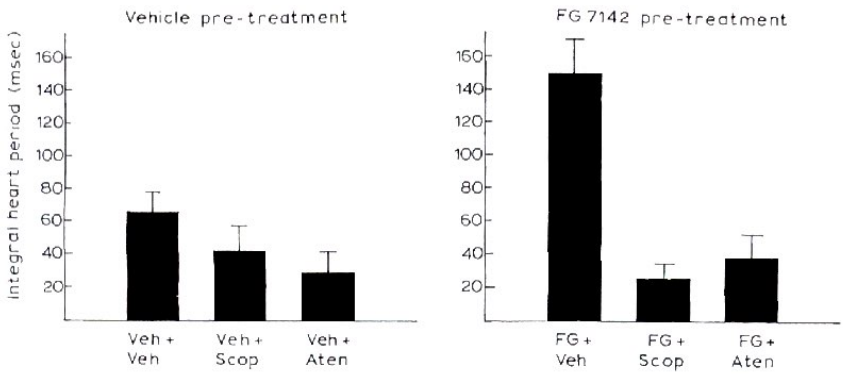

Fig. 2. Effects of FG 7142 or vehicle pretreatment and autonomic pharmacologic blockade on the integral area under the poststimulus acceleratory heart period response are illustrated ($n = 6$ in each panel). The data portray the significant attenuation of the poststimulus response by both atenolol and scopolamine after either vehicle or FG 7142 pretreatment.

than after vehicle (Time Block × Drug interaction: $F_{2,22} = 5.24$, $P < 0.013$; Vehicle post-stimulus block 1 vs. FG 7142 poststimulus block 1: $P < 0.01$).

3.3. Effects of autonomic blockade on basal cardiovascular measures

Under quiescent basal conditions, heart period in the adult rat is under extensive vagal control [41,56]. Consistent with this fact, vagal blockade by scopolamine resulted in a substantive decrease in basal heart period (mean baseline heart period: 170.0 ± 7.5 ms; mean scopolamine heart period: 131.7 ± 2.1 ms). This was revealed by a significant main effect of Scopolamine ($F_{1,5} = 36.61$, $P = 0.002$) and a significant Time × Scopolamine interaction ($F_{1,5} = 105.79$, $P < 0.001$) that reflected the difference between pre- and postscopolamine mean heart period. Conversely, although lengthening heart period somewhat, sympathetic blockade by atenolol yielded no significant effect on basal heart period (mean baseline heart period: 181.2 ± 9.2 ms; mean atenolol heart period: 192.7 ± 5.2 ms; Ps > 0.3).

Although the sympathetic innervation exerts predominant control over vascular smooth muscle via α-adrenergic receptors, the β_1 antagonist employed in the present study would be expected to have minimal effect on the vasculature. Consistent with these considerations, neither scopolamine nor atenolol significantly altered mean blood pressure (all Ps > 0.2).

3.4. Effects of autonomic blockade on phasic cardiovascular responses

Because the response to the auditory stimulus was sustained throughout the poststimulus interval, analyses of the effects of autonomic blockade were performed using the integral areas (in ms) under the entire poststimulus response interval[2].

Effects of autonomic blockade on control responses. Blockade of β_1-adrenergic receptors by atenolol resulted in a significant reduction in the reactive heart period response following vehicle pretreatment (Atenolol main effect: $F_{1,5} = 9.36$, $P < 0.03$; Fig. 2). Thus, the cardioacceleratory response observed after vehicle pretreatment was at least partially attributable to sympathetic activation. Similarly, scopolamine methylnitrate also revealed a significant reduction of the cardioacceleratory response (Scopolamine main effect: $F_{1,5} = 23.40$, $P = 0.005$; Fig. 2). Taken together, these data suggest that the cardiac response following vehicle pretreatment was characterized by sympathetic activation coupled with a reciprocal vagal withdrawal (Fig. 2).

As noted in the methods, the results of the separate autonomic blockades provide estimates of the independent contributions of the autonomic branches to the observed heart period response. Subtractive or residual methods are typically used to derive estimates of sympathetic and parasympathetic contributions to target organ response (e.g. ref. 24). However, the use of both subtractive and residual estimates of autonomic contributions to a target organ response to derive an estimate of bias in blockade data is relatively new [6]. The estimates for each autonomic branch as derived from blockades using the methods outlined by Berntson et al. [6] are illustrated in Fig. 3. Also illustrated are the separate functions derived from the two blockade conditions. Discrepancies between the residual and subtractive estimates and the mean

[2] As with analyses of the effects of FG 7142, heart period integral area measures were log transformed to normalize the data. Ancillary analyses on untransformed integral areas produced results comparable to those of the log transformed integrals.

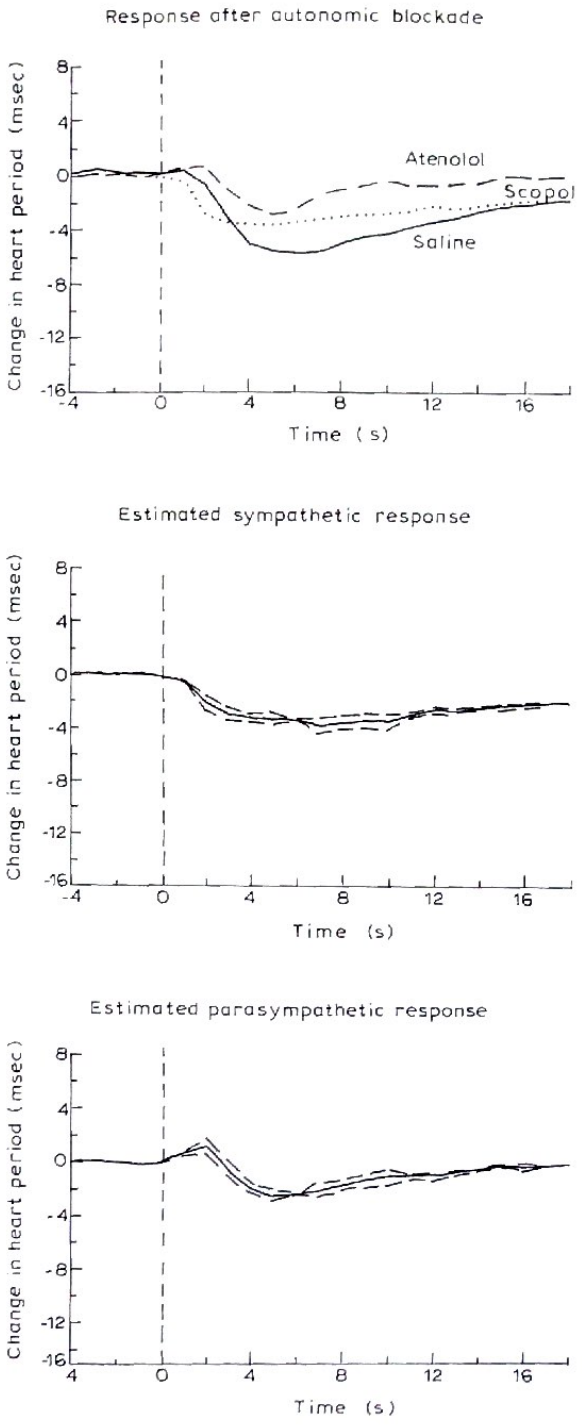

Fig. 3. Top panel: stimulus evoked heart period responses following vehicle pretreatment and administration of either vehicle, β_1-adrenergic blockade (atenolol), or muscarinic blockade (scopolamine). Bottom 2 panels: sympathetic and parasympathetic estimates, and corresponding validity ranges derived by the quantitative methods of Berntson et al. [6]. The validity ranges around the mean sympathetic and parasympathetic estimates reveal minimal discrepancies from the estimated autonomic contributions to the heart period response. The dashed vertical line indicates stimulus onset and only the final 4 s of the prestimulus period are shown for clarity.

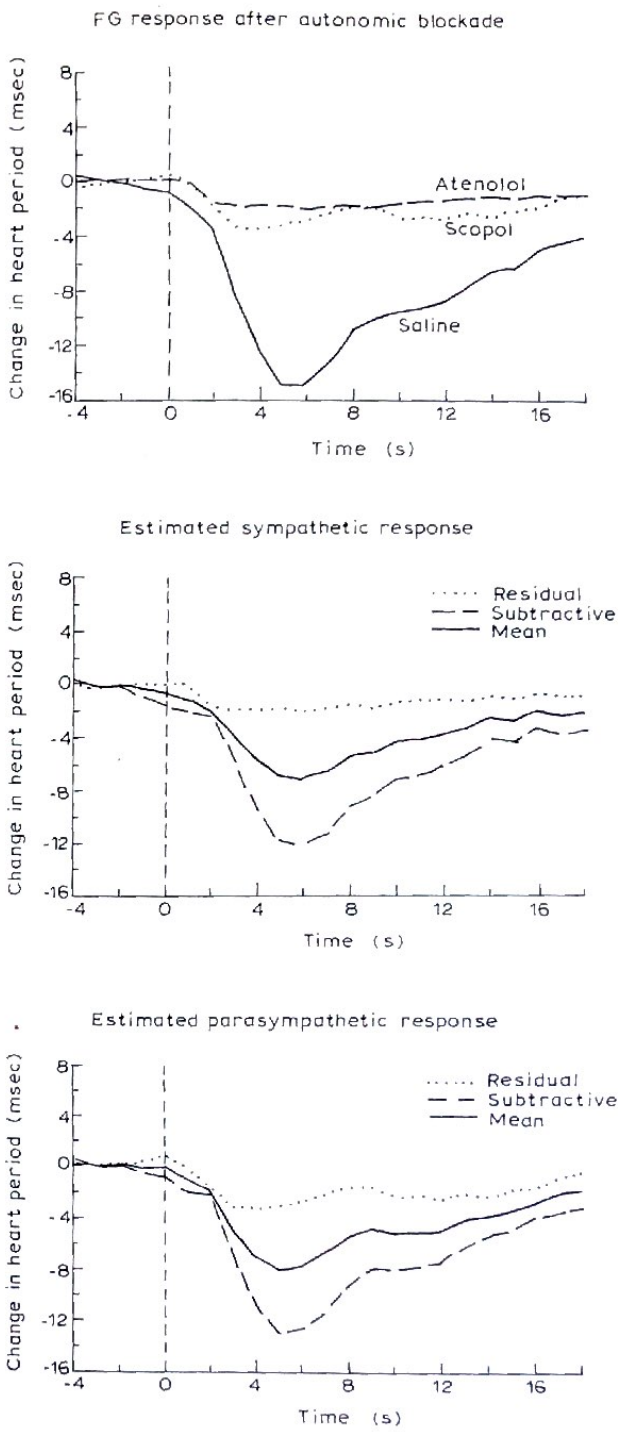

Fig. 4. Top panel: stimulus evoked heart period responses following FG 7142 pretreatment and administration of either vehicle, β_1-adrenergic blockade (atenolol), or muscarinic blockade (scopolamine). Bottom 2 panels: sympathetic and parasympathetic estimates, and corresponding validity ranges derived by the quantitative methods of Berntson et al. [6]. The validity ranges around the mean sympathetic and parasympathetic estimates demonstrate considerable discrepancy from the estimated autonomic contributions to the heart period response. The dashed vertical line indicates stimulus onset and only the final 4 s of the prestimulus period are shown for clarity.

estimate for each autonomic division represents the aggregate systematic error associated with incomplete autonomic blockade, interactions between the autonomic divisions, reflexive effects of single blockades, and non-

selective effects of the blockade agents. For vehicle-pretreated subjects, the validity ranges derived from selective blockade and illustrated by the residual and subtractive estimates around the mean sympathetic and parasympathetic responses suggest that derived autonomic estimates closely approximate the sympathetic and parasympathetic contributions to the heart period response.

In contrast to heart period, analyses revealed no effect of either scopolamine or atenolol on the depressor or pressor responses to the auditory stimulus in vehicle-pretreated subjects, either for the first poststimulus block or for the entire poststimulus period (all $Ps > 0.1$).

3.5. Effects of autonomic blockade on response after FG 7142 pretreatment

Similar to the results after vehicle pretreatment, atenolol significantly attenuated the response to the auditory stimulus following FG 7142 pretreatment (Fig. 2), effectively eliminating the cardioacceleratory response. This is consistent with an FG 7142-induced increase in sympathetic reactivity. However, muscarinic blockade also nearly completely abolished the stimulus-evoked cardiac response (Figs. 2 and 4). Sympathetic and parasympathetic response templates, and corresponding validity estimates were derived using the methods outlined above from the independent activities of the autonomic branches under atenolol and scopolamine blockade [6]. As is apparent in Fig. 4, notable discrepancies were apparent between the subtractive and residual autonomic estimates, suggesting a low validity of these analyses after FG 7142 pretreatment. These results suggest a severe bias or confound in the effects of autonomic blockade under FG pretreatment which is not apparent after vehicle pretreatment. We will return to the issue of this apparent confound in the discussion.

Neither depressor nor pressor responses to the auditory challenge stimulus were altered following autonomic blockade after FG 7142 pretreatment (all $Ps > 0.1$).

4. Discussion

In freely moving rats the benzodiazepine receptor partial inverse agonist FG 7142 modestly increased basal heart period (decreased rate), but did not alter mean blood pressure. More notably, FG 7142 significantly increased the reactive cardioacceleration to a moderate intensity auditory challenge stimulus.

The observed increase in basal heart period following FG 7142 contrasts with findings of decreased heart period (increased rate) after inverse agonist (β-CCE or FG 7142) treatment in primates [13,35,45]. It is not clear if these differences represent species-typical responses, or are a result of procedural variations. The lack of effect of atenolol on the basal heart period reported here suggests that the modest increase in baseline heart period (rate decrease) after FG 7142 pretreatment likely arose from minimal increases in tonic parasympathetic outflow to the heart. Such a result is consistent with evidence of a converse, vagolytic effect of BZR agonists which modulate chloride conductance at the GABA/BZ receptor complex in a direction opposite that of the inverse agonists [1,12,17].

In contrast to the modest effects of FG 7142 on basal heart period, the reactive tachycardic response to a challenge stimulus was enhanced after FG 7142 relative to control conditions. This result is in keeping with increased stress reactivity following inverse agonist administration, as revealed by increased release of corticosteroids and plasma catecholamines [13,35,37,45]. Moreover, the enhanced reactivity after FG 7142 may account for previously reported decreases in basal heart period (increased rate; e.g. refs. 18, 35) because these studies were run under at least mildly stressful conditions (e.g. drug administration to humans in a laboratory or monkeys tested in a restraint chair). Thus the basal cardiovascular measures of previous studies may partially reflect reactive responses. These results serve as an important reminder that modulations of GABAergic transmission by the BZR ligands are made against a background of tonic GABAergic activity. Only under minimally evocative experimental conditions will administration of BZR ligands reveal the effects of modulation of true resting GABAergic function.

To characterize further the reactive cardiac response to the auditory stimulus in the present study, parasympathetic and sympathetic cardiac innervations were pharmacologically blocked by scopolamine methylnitrate and atenolol, respectively. Sympathetic activation in response to the challenge stimulus after vehicle pretreatment was inferred from the significant decrease in magnitude of the cardioacceleratory response after adrenergic blockade with atenolol. In addition, muscarinic blockade with scopolamine similarly attenuated the cardioacceleratory response, suggesting that the sympathetic activation was coupled with a concurrent vagal withdrawal. These inferences are also supported by the minimal derived estimates of error for the sympathetic and parasympathetic estimates of response under vehicle conditions. Taken together, these data suggest that the auditory stimulus evoked a reciprocal sympathetic mode of chronotropic control of the heart.

Pharmacologic blockade of sympathetic and parasympathetic actions on the heart was similarly used to delin-

eate the contributions of each autonomic branch to the cardiac response after FG 7142. However, Fig. 4 (upper panel) revealed that both scopolamine methylnitrate and atenolol almost completely blocked the cardioacceleratory response. This result is generally consistent with a reciprocal mode of sympathetic activation and parasympathetic withdrawal. However, if sympathetic activation contributed significantly to the cardioacceleratory response, as is suggested by the atenolol blockade results, then a considerable cardioacceleratory response should also be observed after scopolamine blockade. Similarly, if parasympathetic withdrawal were a significant contributor to the cardioacceleratory response, then substantial cardioacceleration should still be manifest after atenolol blockade. Thus, the autonomic blockade results do not permit a simple interpretation. Moreover, as is apparent from Fig. 4, the validity (error) estimates deviate considerably from the mean sympathetic and parasympathetic contributions. The discrepancies illustrated by the autonomic blockade results and the error estimates could arise from several sources, including: (a) incomplete autonomic blockade, (b) an interaction between the autonomic divisions that is eliminated by blockade of one division, (c) a "floor effect" manifested after the basal decrease in heart period under scopolamine blockade, (d) reflexive alterations in activity of the unblocked division with single autonomic blockade, or (e) a confounding of the effects of FG 7142 and one of the blocking agents. As noted above, use of residual or subtractive methods alone to estimate autonomic contributions to the cardiac response would preclude simple inferences. The validity estimates derived in the present analysis, however, provide an illustration of the magnitude of these discrepancies in relation to the magnitude of the cardiac response.

Firstly, it appears that the biases in the sympathetic and parasympathetic estimates after FG 7142 did not result from incomplete autonomic blockade or autonomic interactions. Incomplete autonomic blockades would yield residual overestimates of autonomic control, and subtractive underestimates that approached zero. Fig. 4 reveals that the opposite pattern was observed, suggesting that the low validity of the sympathetic and parasympathetic estimates did not arise from incomplete blockades. Similarly, elimination of an inhibitory interaction like accentuated antagonism in which increasing sympathetic activation produces progressively longer heart periods for a given level of parasympathetic activation would yield a cardioacceleration under unblocked (saline) conditions that was smaller than the acceleratory response under either sympathetic or parasympathetic blockade. Fig. 4 reveals that exactly the opposite pattern obtained. Moreover, autonomic interactions are most apparent under conditions of high activation of both divisions, rather than at the moderate basal levels of activity observed in the present study [27,29,47]. Most importantly, these interaction effects have been demonstrated in the literature using heart rate change scores, but the short-term autonomic interactions appear to be minimal when heart period is employed as the metric [42,47]. Finally, potential "floor effects" on heart period after scopolamine blockade could limit further tachycardic responses. However, the vehicle response to the challenge stimulus was even larger in magnitude than that after FG 7142 pretreatment suggesting that no physiological boundary was attained.

Finally, the disparity between the validity intervals and estimates of the sympathetic and parasympathetic contributions to the heart period response did not appear to arise from baroreflexive alterations in activity of the unblocked division under selective blockades. Basal blood pressure was unaltered by FG 7142, and reactive blood pressure responses were not affected by autonomic blockade. Moreover, no baroreflexive alterations were apparent with autonomic blockades under control conditions. Although one cannot rule out the possibility of other such reflexive alterations in the present study, the baroreceptor reflex represents one of the most prominent reflexes that is likely to impact on cardiac chronotropic state. Hence, the low validity of sympathetic and parasympathetic estimates after FG 7142 appears to reflect a specific interaction between the BZR inverse agonist and the autonomic blockers.

Recent studies have suggested that the quaternary ammonium derivatives may not remain as primarily peripheral as once believed [32]. For example, both microdialysis and cup technique studies have reported increases in cortical acetylcholine following administration of the quaternary compound, atropine methylbromide that were only 2–4 times less than increases induced by atropine sulfate [49,52]. Moreover, several studies employing cognitive tasks using both scopolamine (or atropine) and its methylated compound do not demonstrate differences across these compounds (e.g. ref. 19).

Thus, it is possible that one effect of scopolamine methylnitrate was the alteration of a central cholinergic action of FG 7142. In this regard, microdialysis evidence suggests that both selective (ZK 93426) and partial (FG 7142) BZR inverse agonists enhance physiological release of cortical acetylcholine [33,34], and central cholinergic stimulation is known to yield sympatho-excitatory effects [10]. FG 7142 may have potentiated the cardioacceleratory response by this central cholinergic action, an action that was blocked by scopolamine. Consistent with this interpretation, studies by DiMicco and colleagues demonstrate that GABAergic antagonists microinjected into posterior hypothalamus decrease heart period (increase rate), increase blood pressure and increase splanchnic

sympathetic nerve activity similar to the phasic effects of FG 7142 observed in the present study, and in contrast to the effects of GABA agonists [28,54].

On the whole, these data support several conclusions concerning the cardiovascular effects of FG 7142 and suggest possible sites of action of the BZR inverse agonists. First, although basal heart period was modestly increased (rate decreased) in response to FG 7142 administration, tachycardic responses to a challenge stimulus were enhanced relative to vehicle conditions. These results caution against sole reliance on tonic cardiovascular measures in assessing pharmacologic agents such as the BZR ligands, and illustrate the importance of non-evocative experimental settings when evaluating the effects of BZR ligands. In addition to the assessment of cardiovascular end-organ measures, autonomic blockades demonstrated that under control conditions, the neural origins of a response to an auditory challenge stimulus could be characterized by a reciprocal sympathetic activation and vagal withdrawal. Finally, the apparent scopolamine interaction with the effects of FG 7142 raises the possibility of a central cholinergic action of FG 7142 that is related to its cardiovascular effects. Further studies of the postulated central cholinergic activation by FG 7142 and its relationship to physiological and psychological stress responses will provide an even better assessment of the "anxiogenic" hypothesis of BZR inverse agonist function.

Acknowledgements

This work was supported by a Sigma Xi Grant-in-Aid of Research and a Graduate Student Alumni Research Award.

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