VISCERAL REFLEX RESPONSES FOLLOWING RIGHT INTRA-ATRIAL INJECTION OF PHENYLDIGUANIDE IN RATS*

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Summary: 1. Intra-atrial injection (right atrium) of pdg in nembutal anaesthetised rats produced bradycardia, hypotension and apnoea followed by hyperpnoea. In very lightly anaesthetised rats, injection of pdg close to the aortic valves produced similar responses and these responses disappeared on maintaining the animals in well-anaesthetised condition.

2. Administration of pdg either into the cerebral circulation or into cerebral ventricles did not produce bradycardia and apnoea.

3. The afferent apthway for these autonomic responses runs in vagus nerve, as shown by experiments before and after bilateral vagotomy.

4. The electrical activity of both expiratory and inspiratory muscles was inhibited during end-expiratory apnoea phase following injection of pdg into the right atrium.

5. Glycine, administered centrally or intravenously, exhibited blockade of pdg induced autonomic responses for more than forty minutes.

Key words: phenyldiguanide, glycine, type J pulmonary receptors, bradycardia, hypotension, apnoea

INTRODUCTION

Reflex responses of type J receptors (19) have been studied by several investigators using phenyldiguanide (pdg) as a chemical tool to activate these non-medullated afferent nerve terminals (1, 21, 22, 24). Reflex depression of skeletomotor as well as certain visceral function on activation of these type J receptors are well eatablished in cats (1, 7, 14, 27, 28) and also in dog fish (24). The visceral reflex responses to pdg were also reported in rabbits (2, 12,25). In order to appraise the functional significance of these visceral and somatic reflex responses elicited by stimulation of type J receptors, it is necessary to establish these reflexes in various other species. The present paper deals with the few functional properties of the visceral reflexes elicited by pdg in rats.

Though type J receptors were held responsible as the sole sensory mechanism for both bradycardia and apnoea following right atrial injection of pdg (18), there seems to be no direct evidence that the observed visceral responses are not due to the result of direct central action of pdg. Therefore, in the present paper, whether pdg has any capability to evoke these visceral responses through its central action, has also been evaluated.

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MATERIALS AND METHODS

Adult albino rats (Wister strain) weighing between 200 and 300 *gm* were anaesthetised with pentobarbitone Na (4 *mg*/100 *gm*/i.p.). Following tracheal cannulation, a catheter was introduced through the right external jugular vein, so that its tip lay in the right atrium. In a few rats, a second cannula was passed through left common carotid artery so as to reach close to the arch of arota. The positions of both the cannlae were confirmed by postmortem examination. In order to inject pdg close into the cerebral circulation, the peripheral end of right common carotid artery was also cathetarised.

The central action of pdg on cardiovascular and respiratory system was examined by direct injection or slow infusion of the drug into the cerebral ventricle. For this purpose, a ventricular cannula was placed stereotaxically into the right lateral ventricle and the cannula was fixed on the skull with dental acrylic cement (Dental Fillings Corporation, England) as described by Feldberg & Saxena (9). The drug was infused through a fine hollow needle inserted through the length of the cannula shaft, but not beyond it. The other end of hollow needle was connected to the microlitre slow injector apparatus through a fine polythene tubing. The rate of infusion was set at 23 μl/min. The placement of ventricular cannula was confirmed by injecting or infusing 0.8% bromophenol blue dye through the hollow needle and the extent of the spread of dye in the cerebroventricular system was noted through postmortem examination. It was observed that the dye stained all the cerebral ventricles and often the ventral surface of the brainstem.

The systemic arterial blood pressure was monitored by means of a mercury manometer connected to the carotid cannula. The respiratory rate and heart rate were recorded simultaneously on 16-channel polygraph (Kaiser, Denmark). The electro-cardiographic recording was made by using standard bipolar limb II lead. The electrical activity from functional multi- or single units of intercostal muscles was recorded by means of bipolar platinum electrodes connected to a preamplifier with 10 KHz to 0.2 KHz frequency response. The preampflier output was displayed on an storage oscilloscope (DISA, Denmark) and was recorded on a photographic paper.

The non-medullated vagal afferents were activated chemically by quickly injecting 10 μg of pdg dissoved in 0.1 *ml* of normal saline (0.9%). Repeated injections of the drug did not produce tachyphylaxis in cats (7,22). However, in these experiments we have restricted the maximum number of injections to ten for each rat studied. For cerebroventricular administration of drug, pdg was dissolved in 30 μl of artificial c.s.f. and this volume was kept constant with varying doses of the drug used.

RESULTS

A. pdg injection into right atrium:

Following an injection of pdg (10 μg) into right atrium, a depression of heart rate, blood

pressure and respiratory rate lasting for several seconds was observed. As a control study, when 0.1 *ml* of 0.9% saline was injected through the same route, no effects on heart rate, blood pressure and respiration occurred.

(*i*) *Cardiovascular responses*: Fig 1 shows that approximately 0.6 sec after the drug injection, the heart rate was reduced to about 20% with peak depression between 2 and 4 sec and which gradually recovered to the control values after about 10 sec. In case of blood pressure, it was reduced to 91% of the control value and the peak depression (about 84%) was between 12 and less than 30 sec, followed by a slow recovery which was 100% at about 210 sec after the injection. Table I shows the results on heart rate of 11 different animals following pdg admini-

TABLE 1: Effect on heart rate and respiration after intra-atrial injection of pdg.

Experiment no. & serial no. of injections		*Rate as % of control*		*Approximate duration (sec.)*	
		Heart rate	*Respiratory rate*	*Heart rate response*	*Respiratory response*
R-8	(i)	35	20	1.8	6.5
	(ii)	46	42	1.6	4.5
R-10	(i)	32	138*	1.9	4.0
	(ii)	40	140*	1.6	4.6
R-11	(i)	37	28	5.1	3.5
	(ii)	51	21	1.0	5.0
R-13	(i)	60	27	5.8	6.0
	(ii)	68	13	5.4	5.0
	(iii)	75	19	8.0	6.5
R-14	(i)	48	140*	10.4	6.8
R-17	(i)	46	20	8.0	3.0
	(ii)	52	30	5.1	6.0
	(iii)	35	60	4.0	4.3
R-18	(i)	53	15	4.9	4.5
	(ii)	20	10	8.0	9.0
	(iii)	70	18	3.2	6.0
R-19	(i)	42	132*	1.2	2.5
	(ii)	28	126*	2.4	6.5
R-21	(i)	35	22	3.5	5.6
	(ii)	48	37	2.7	5.0
	(iii)	57	45	1.5	4.5
R-22	(i)	45	30	3.1	4.1
	(ii)	20	25	4.0	5.7
R-23	(i)	37	18	4.8	5.5

Control heart rate and respiratory rate were taken as 100%
*Only hyperpnoea was observed

stration. It can be observed that the reduction of heart rate, calculated around the point of p ak reduction, varied between 75 and 20% of the control and the time taken to reach this peak response varied between 1.07 and 2.40 sec after the drug injection.

Generally the hypotension occurred after a latency of 2 to 4 sec. The maximum fall in blood pressure occurred between 10 and 70 sec after the injection and it ranged from 90 to 40% of the control value. The degree of hypotension was considerably dependent on the initial blood pressure level, that is, with the higher initial blood pressure, the fall was also greater.

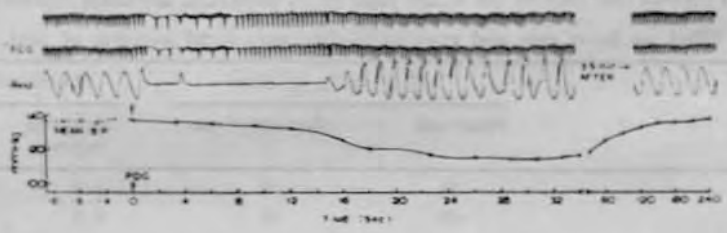

Fig. 1: Shows bradycardia, apnoea and hypotension following right intra-atrial injection of 10 µg pdg. The blood pressure was recorded kymographically from the same animal and was plotted against the same time scale. Top record — heart rate; Middle record — respiration; Bottom record — blood pressure.

(ii) *Respiratory responses*: Fig. 1 shows that approximately 0.6 sec after intra-atrial pdg injection, the respiratory rate was reduced to about 10% of the control value and the peak depression was between 1 and 8 sec. Then it gradually recovered after about 10 sec. Respiratory effects varied from absolute respiratory arrest in end-expiratory phase to occurrence of hyperpnoea (Table I). In ten out of eighteen experiments, the response was a mixed type; in some cases the apnoea preceded tachypnoea, and in others, just the reverse occurred. In six cases, the only response observed was hyperpnoea and in two cases it was only apnoea. It will appear from Table I that the respiratory rate changed from 190 to 20% of control and the approximate duration of response varied between 1.30 to 7.3 sec. The period of recovery for bradycardia, hypotension apnoea and hyperpnoea showed considerable variation. Tachyphylaxis did not develop following repeated injections of pdg.

Multiunit discharge from the inspiratory and expiratory muscles was very much depressed during apnoea and soon recovered to control value as apnoea faded off (Fig. 3). Thus it indicated that both expiratory and inspiratory muscles activity were inhibited during reflex apnoea.

B. pdg injection into aorta:

In order to determine the part played by the non-pulmonary receptors (15, 17, 19) in producing apnoea and bradycardia, pdg (10 μg on 0.1 *ml* saline) was injected abruptly, close to the aortic valves and the effects on the respiration and heart rate were followed. The results are given tn Table II.

(*i*) *Cardiovascular responses:* There occurred a reduction of heart rate ranging from 60 to 44% of the control as compared to 43 to 25% due to intra-atrial injection.

(*ii*) *Respiratory responses*: From the Table II it appears that the effect of *intra-atrial* injection of pdg on respiration essentially consisted of a trasient apnoea followed by hyperpnoea. The maximum extent of depression of respiratory rate on right atrial injection varied from 36 to 50% of control value and the increase of hyperpnoea from 160 to 130% of the control. On the other hand, the extent of apnoeic phase and the increase of hyperpnoeic period following *aortic* injection ranged from 100 to 90% and 160 to 137% of the control rate respectively. The period of hyperpnoea was slightly longer and the apnoea was almost absent on aortic injection of pdg. It can be concluded tentatively that the systemic and coronary circulation of pdg plays an insignificant role in causing apnoea.

TABLE II: A comparison of intra-aortic and intra-atrial injection of 10 μg of pdg on heart rate and respiration rate.

Rat No.	*Intra-Aortic Injection*				*Intra-Atrial Injection*			
	Heart rate		*Respiration rate*		*Heart rate*		*Respiration rate*	
	Peak response as % of control	*Duration (approx.) in sec.*	*Peak response as % of control*	*Duration (approx.) in sec.*	*Peak response as % of control*	*Duration (approx.) in sec.*	*Peak response as % of control*	*Duration (approx.) in sec.*
1	50	3.3	A nil	—	43	4.65	A 48	1.6
			H 160	6.6			H 160	12
2	39	3.3	A nil	—	25	4.5	A 40	12
			H 140	6.0			H 160	2.5
3	52	2.5	A 90	1.5	34	3.1	A 36	4.3
			H 137	5.0			H 149	3.6
4	44	2.0	A nil	—	30	4.2	A 45	3.5
			H 155	4.1			H 130	2.9
5	60	3.0	A nil	—	36	3.9	A 50	4.5
			H 140	3.0			H 165	5.6

A = apnoea; H = hyperpnoea

It has been also observed that the reflex responses following *intra-aortic* injection occurred in those animals which are under light anaesthesia. Following the anaesthesia deeper in the same animal, reflex responses due to intra - aortic injection disappeared but those due to intra-atrial injection remained.

C. Effect of bilateral vagotomy:

If the observed visceral responses on right atrial injection of pdg are produced as a result of stimulation of sensory nerve endings in pulmonary bed, then vagotomy should abolish these responses. This was confirmed in four experiments, one of which is illustrated in Fig. 2. The ECG and respiratory rate were recorded and pdg was injected into right atrium. The continuous line in Fig. 2A represents the reduction of respiratory rate to 10% of control value within 1.0 sec after the injection and it recovered only after 8 sec. The continuous line in Fig. 2B shows the reduction of heart rate to 22% of control value with slow recovery. Bilateral vagotomy (interrupted line in both Fig. 2A & 2B) show that pdg no longer depressed the respiration and heart rate. It can therefore be concluded that pdg produced the observed visceral responses primarily through its action on vagal afferants present in the lungs.

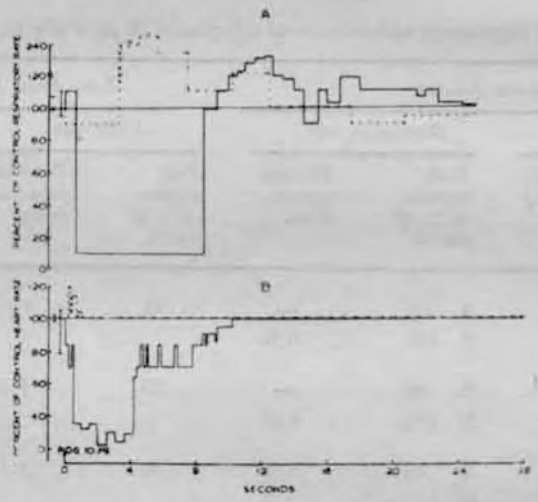

Fig. 2: Shows abolition of pdg-induced apnoea and bradycardia after bilateral vagotomy. Solid line — before vagotomy; Interrupted line — after vagotomy, Dose — 10 μg. For explanation see the text.

D. Intracerebroventricular administration of Pdg :

The major cause of hypotension in cat is due to bradycardia produced by injection of pdg. Since bilateral vagotomy interrupts both the afferents as well as efferent innervation to the heart, it can not preclude the possibility that the central action of the drug might contribute to the bradycardia. This possibility was examined by quickly administering or infusing the pdg (10 μg in 50 μl artificial c.s.f. solution) into right lateral cerebral ventricle through a chronically placed cannula, while ECG, blood pressure and the respiration were being recorded. It was observed that pdg did not produce any change in heart rate, blood pressure and resporation on cerebroventricular injection of drug upto the doses of 50 μg.

E. Effect of glycine on reflex cardiovascular and respiratory responses to pdg:

The evidence that glycine is a inhibitory synaptic transmitter in both spinal cord and medulla of cats and rats is now substantial. Therefore, experiments were carried out to examine whe-

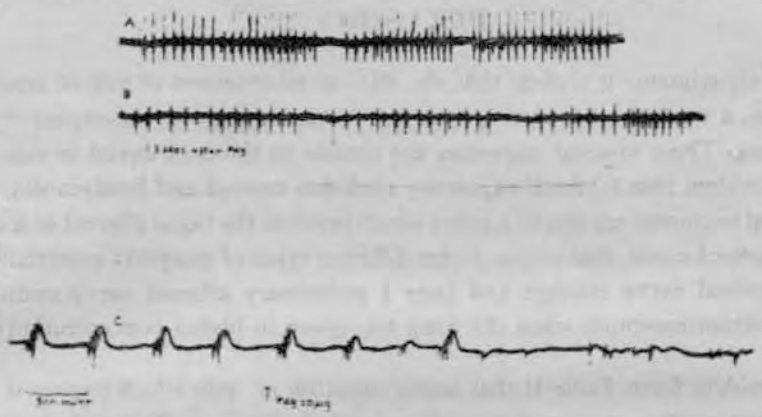

Fig. 3: Shows inhibtion of motor unit potentials from intercostal respiratory muscles after pdg injection.
A — motor unit potentials from expiratory muscles before pdg.
B — 1.5 sec after pdg. Note diminution of potentials.
C — motor unit potentials from inspiratory muscles. Note profound inhibition of potentials after pdg injection. The record was taken from the same animal.

ther reflex visceral responses to right-atrial injection of pdg can be altered by raising the concentration of glycine in the brain. For this purpose, 50 μg of glycine in 50 μl artificial c.s.f. was infused slowly into the right lateral carebral ventricle (icv) of four rats, and the cardiovascular and respiratory responses to pdg were tested at different time intervals over an hr. A considerable blockade of the reflex responses was observed after icv glycine infusion in all rats, one of which illustrated in Fig. 4. It will be noted from the illustration, that reflex bradycardia was reduced

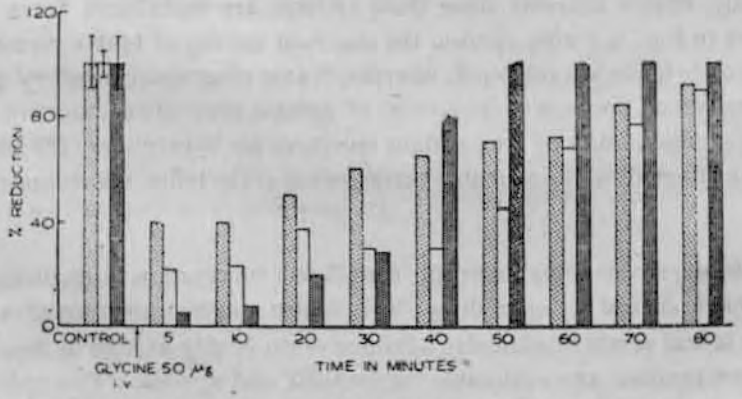

Fig. 4: Shows the blocking effect of cerebro-ventricular injection of 50 μg of glycine on visceral responses elicited by pdg.
Stippled Bar — arterial blood pressure; White Bar — respiration; Dense stippled Bar — heart rate.

to 5%, apnoea to 22% and hypotension to 40% of their control values, five minutes after glycine administration. At the end of 50 min, the bradycardia responses recovered to 100%, hypotension to 70% but the apnoea was still 45% of its control value.

DISCUSSION

From the experiments it is clear that the intra-atrial injection of pdg in nembutal anaesthetised rat produces a marked bradycardia, hypotension and apnoea in end-expiratory phase followed by hyperpnoea. These visceral responses are similar to those observed in cats (5,14). From Fig. 2 it is also evident that bilateral vagotomy abolishes apnoea and bradycardia. This indicates that these visceral responses are due to a reflex which involves the vagal afferent as a sensory mechanism. It is however known, that pdg activates different types of receptors essentially chemoreceptors, gastro-intestinal nerve endings and type J pulmonary afferent nerve endings (15, 17, 19) and also lung irritant receptors when the drug was given in higher concentration (2).

But it is evident from Table II that aortic injection of pdg which bypassed the pulmonary circulation did not produce apnoea but produced only hyperpnoea and slight bradycardia. These observations suggest that the apnoea and part of the bradycardiac response are due to the stimulating actions of pdg on vagal afferent nerve terminals from lungs. These vagal afferents from lungs are essentially classified into three main groups: (i) pulmonary stretch, (ii) type J afferents and (iii) afferents from irritant receptors (2). Pdg induced visceral reflex responses in rat (Fig. 1) shows a striking similarity with the visceral reflex responses elicited by stimulation of type J pulmonary afferents (18, 19). Essentially the type of apnoea (Fig. 3) where the gamma activity of both expiratory and inspiratory intercostal muscles was shown to be inhibited (27), supports the view that the sensory mechanism for both end-expiratory apnoea and bradycardia probably results from the stimulation of type J pulmonary receptors with pdg. These reflex responses are not likely due to activation of pulmonary stretch receptors on two grounds: firstly, pdg does not activate pulmonary stretch afferents since these endings are medullated nerve fibres (16,20); secondly as shown in Fig. 3, during apnoea, the electrical activity of both expiratory and inspiratory intercostal muscle fibres was inhibited; whereas, in case of apnoea caused by pulmonary stretch receptors, there was an increase in the activity of gamma fibres of expiratory intercostal (8,26). The reflex effects of stimulation of lung irritant receptors are hyperpnoea (25) and hypertension (33), thus excluding them from the probable participation in the reflex visceral responses produced by pdg.

Since the bilateral vagotomy interrupts the efferent innervation to the heart also, it cannot preclude the probable central action of drug which in turn may be instrumental in causing bradycardia. But the lateral cerebroventricular administration of pdg as high as five times the intra-atrial dose did not produce any noticeable bardycardia and apnoea. This indicates that these autonomic responses observed on right-atrial injection of pdg are not due to the action of drug on the structures located on the surface of the carebral ventricles and on the ventral surface of

the brainstem. One might however reasonably argue that this route of administration may not provide the access for the drug to act on the target neuronal site/sites, if they are deeply located in the brainstem. But also, when the drug was injected into cerebral circulation through the common carotid artery, it did not produce either bradycardia or apnoea. This finding along with the previous one strongly support the view that the observed bradycardia and spnoea following right atrial injection of pdg are mainly the reflex responses elicited by the action of the drug on the non-medullated pulmonary afferents traversing through vagus nerves and the central action of the drug plays no role in causing these autonomic responses.

The neural mechanisms responsible for reflex depression of visceral responses may involve post-synaptic or pre-synaptic inhibition, or disfacilitation, or all together. Many electrophysiological studies indicate that certain aminoacids specially glycine and glutamate may be acting as transmitters in central nervous system (4). The synaptosomal distribution of glycine in rat brain further supports this view (32). It is evident from Fig. 4 that lateral cerabroventricular injection of glycine blocked the autonomic reflex responses elicited by stimulation of non-medullated pulmonary afferents for more than forty mintues. This finding probably indicates that glycine, through some unknown mechanism, depresses the central neuronal pathway that mediate these visceral responses. This blockade of reflex responses with glycine has also been observed following its intravenous injection of the same dose as that of cerebroventricular route. This blockade on systemic injection may result from the quick passage of glycine across the blood-brain barrier to the central nervous system. There is evidence that the glycine crosses the blood-brain barrier after systemic administration at relatively faster rate in rat brain (23, 30). Such central action of glycine has a'so been observed in other experimental situations. For example, peripherally administered glycine relieves experimental hindlimb rigidity (29), protects against drug-induced convulsions (31) and potentiates barbiturate anaesthesia (13,31).

Since it has been suggested that sensation of dyspnoea aroused under certain pathological conditions could be due to stimulation of non-medullated pulmonary afferent nerve terminals (18), and such sensation of dyspnoea can be relieved by vagal nerve block (10,11), it would therefore be worthwhile to examine the idea if systemic administration of glycine can relieve the sensation of dyspnoea under these pathological conditions. It is interesting to mention that in conditions of acidosis and respiratory distress, hyperglycinemia develops which may be of some physiological significance.

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