LIMITATIONS IN THE USE OF CO_2 AS A METHOD FOR STUDYING THE J-REFLEX

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Summary: In order to study the J-reflex, monosynaptic reflexes were recorded from L_7 or S_1 ventral root after stimulation of the posterior biceps, and semi-tendinosus nerve (PBST) from the lower limb in cats anaesthetisized with Pentobarbitone sodium. Intratracheal CO_2 (60 *ml*, 100%) depressed the monosynaptic reflexes, and the depression was comparable to the effects of right atrial phenyl diguanide injection. Bilateral vagotomy did not abolish the response showing that the afferent pathway of this depression does not travel via the vagus nerve. Thus it is concluded that CO_2 cannot be used to study the J-reflex.

Key Words: monosynaptic reflex PDG J-reflex CO_2

INTRODUCTION

In 1969, Paintal redesignated the deflation receptors as the type J pulmonary endings and showed that the natural stimulus for these endings was pulmonary congestion. Deshpande and Devanandan (3) injected phenyl diaguanide (PDG) intra-atrially to activate the type J receptors and found reflex inhibition of monosynaptic reflexes (MSR) from hind limb muscles. of cat. This phenomenon was termed as the J-reflex by Paintal (11). Since muscular exercise caused a rise in pulmonary arterial pressure (5) which may activate the type J-receptors, Paintal put forward the hypothesis that J-reflex may be an important viscerosomatic reflex to terminate the exercise. As the simulation by natural stimulus i.e. Pulmonary congestion to stimulate type J-receptors involves a number of technical difficulties, phenyl diguanide became the drug of choice to study the characteristics of these endings (11). But it was shown that Phenyl diguanide has got tremendous species difference to activate various lung receptors (2). Thus a suitable stimulus to study the J-reflex in various species had to be worked out. Dickinson & Paintal had shown that CO_2 activates type J receptors (4). It was, therefore, of interest to see its role in studying the J reflex.

MATERIALS AND METHODS

Twelve adult cats weighing 2.4 to 5 *kg* were anaesthetised with intraperitoneal injection of Pentobarbitone sodium (40 *mg/kg*). The trachea was cannulated by a glass cannula with side tubes for injecting CO_2 or air. Two polythene catheters were placed, one in the right atrium to inject PDG or saline and the other in the aorta to monitor the blood pressure as well as to atropinise the animal (Atropine Sulphate 1 *mg/kg*). The nerve to the posterior biceps and semitendinosus (PBST) was dissected out carefully and cut close to the muscle. The other branches of the sciatic nerve were cut. Electrical stimulation by rectangular wave pulses of 0.1 msec duration, and 1.5 times the threshold value was given to the PBST nerve every 2 seconds by silver-silver chloride electrodes in order to stimulate all group I fibres (a and b) (10). Monosynaptic reflexes were recorded from the L_7 or S_1 ventral roots after performing laminectomy from L_4 to S_1 segments. Care was taken not to damage the dorsal root or dorsal root ganglia. The reflexes were displayed on the oscilloscope (Tektronix type 422) and were photographed by Cossor camera.

The monosynaptic reflexes were recorded for a period of 200 to 300 seconds to show if there were any linear trend of depression (12). This was followed by a rapid injection of 2 *ml* of PDG (200 *µgm*) into the right atrium and the monosynaptic reflexes recorded till the recovery of the pre-injection control heights were obtained. As a control, 2 *ml* of 0.9% NaCl was injected into the right atrium in place of PDG and the responses were recorded as before.

Sixty *ml* of 100% Co_2 was then introduced through the tracheal cannula in the end-expiratory phase and the effects on MSR were noted. A diagrammatic sketch of the experimental design is given in Fig 1. Equivolumes of air or 100% N_2 (60 *ml*) was also used to see their effects on MSR and served as a control. As a routine, 5-10 minutes gap was given in between each series of experiments and the test trials repeated several times in any one experiment. Following these observations bilateral vagotomy was performed, and after a gap of half to one hour, all the above mentioned series using PDG, saline, CO_2 and air or N_2 (100%) were repeated. The height of each

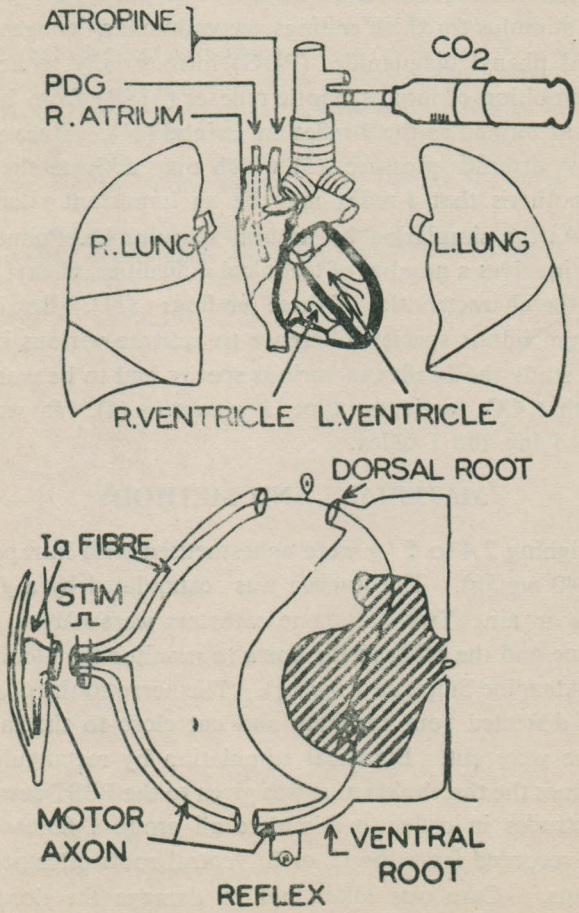

Fig. 1 : Represents the diagrammatic sketch of the experimental design.

control reflex was measured carefully, and the arithmatic mean of the control reflexes was considered as 100%. Now on a graph paper the measured values of the individual reflexes were plotted as percentage of the everage control values. The mean blood presure was maintained above 120 mm of Hg throughout the experiment by continuous intravenous drip of 5% glucose in saline. The body temperature of the animals was maintained at 37°-38°C.

RESULTS

Fig. 2 compares the effect of PDG and saline with that of Co_2 and air on the monosynaptic reflexes recorded from L_7 vental root. The ordinate shows the height of the reflexes presented as percentage of the average control height taken as 100% (represented by the horizontal line).

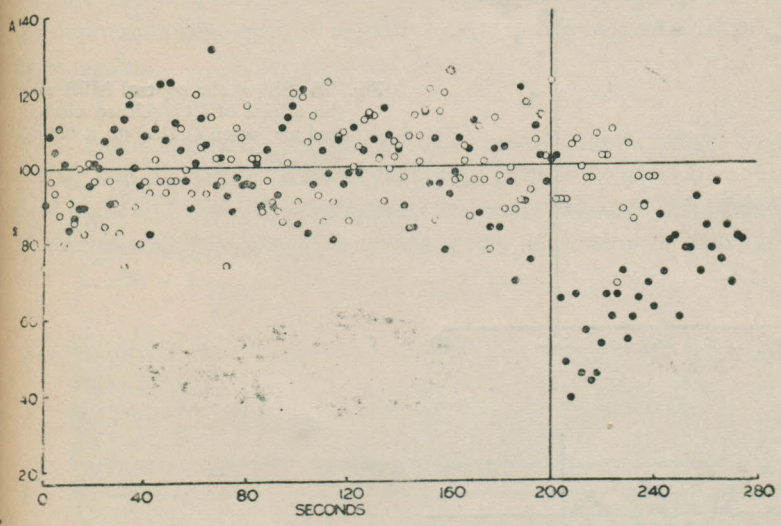

Fig. 2A: Compares the effect of PDG (closed circles) and saline (open circles) on MSR. The point of injection is indicated by the vertical line.

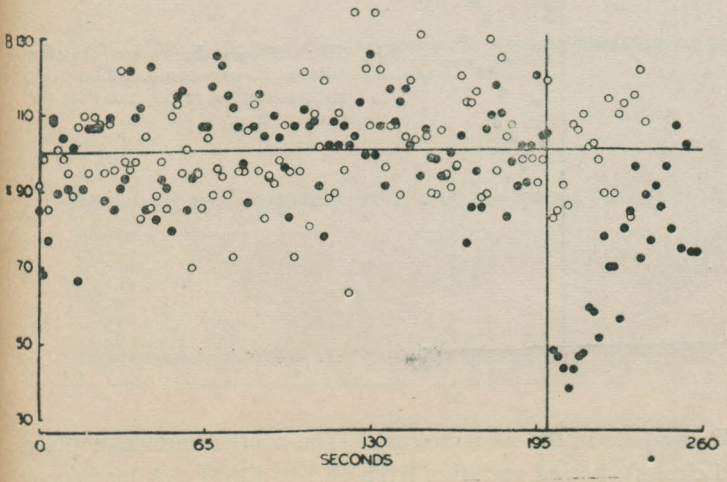

Fig. 2B: Shows the effects of CO_2 (closed circles) and air (open circles) injected at the point of vertical line.

The abscissa shows the time in seconds. The filled circles (Fig 2A) show the control values and the effect of PDG on the monosynaptic reflexes. There is a variation in the control heights ranging from 69% to 120%. The vertical line demarcates the exact point of injection of PDG or saline. PDG produces a depression of the monosynaptic reflexes upto 38.1% (at 8th second). The depression, with gradual recovery persisted for 54 seconds. By contrast saline did not change the control heights (open circles) which also ranged from 73.8% to 125% of the average control value and were quite comparable to the pre-PDG control heights.

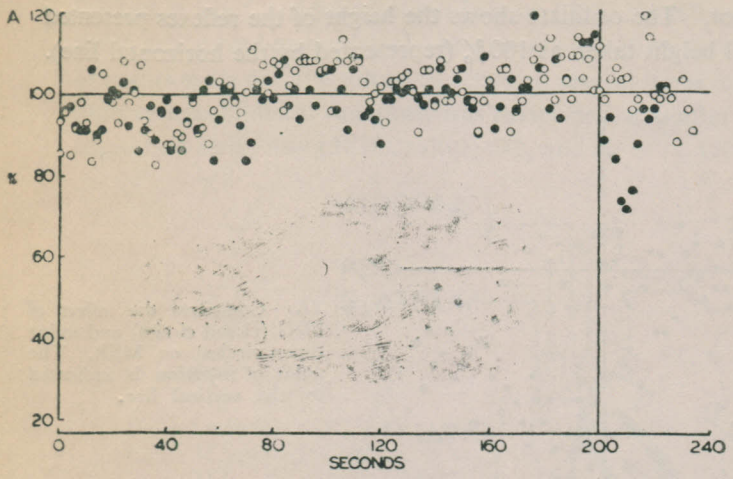

Fig. 3A: Shows the control MSR and the effects of CO_2 (closed circles) and air open circles) on it before vegotomy.

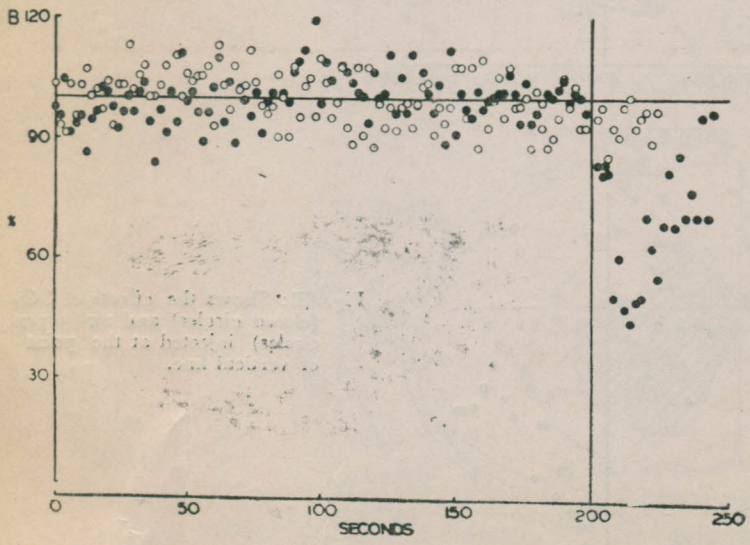

Fig. 3B : Shows the responses after bilateral vagotomy. The test substances were injected at the vertical line.

Fig 2B shows the effects of CO_2 and air in the same animal. The control monosynaptic reflexes before injection of CO_2 (filled circles) showed variation from 66.5 to 125%. After injec-

tion of CO_2 (at the vertical line) the reflexes are depressed upto 37.2% (at the 8th second) which with gradual recovery lasted for 32 seconds. The control values before inejction of air (open circle) range from 69.8% to 136% of the average. Injection of air produced no change in these reflex heights.

Fig. 3 shows the comparison of the effects of the intratracheal injection of CO_2 (filled circles) and air (open circles) before (3A) and after bilateral vagotomy (3B) in the same animal. The reflexes are recorded from S_1 ventral root. CO_2 injection before vagotomy depressed the MSR to 71.2% (Fig. 3A): closed circles which lasted for 20 seconds. Air, as seen in other series, did not bring about any change (Fig. 3A: open circles). Following bilateral vagotomy (Fig. 3B: filled circles) CO_2 depresses the monosynaptic reflexes upto 44.6% (in the 14th seconds) and the depression lasts upto 38 seconds. Air in this case also did not produce any change (Fig. 3B open circles).

Fig. 4 shows the actual records of monosynaptic relexes comrpising respectively the effect of Co_2 and air before bilateral vagotomy [Fig. 4 (1) and (2)] and after bilateral vagotomy [Fig. 4 (3) and (4)]. Table I summarises the results obtained before and after bilateral vagotomy. In all the animals PDG failed to produce any depression of MSR after bilateral vagotomy.

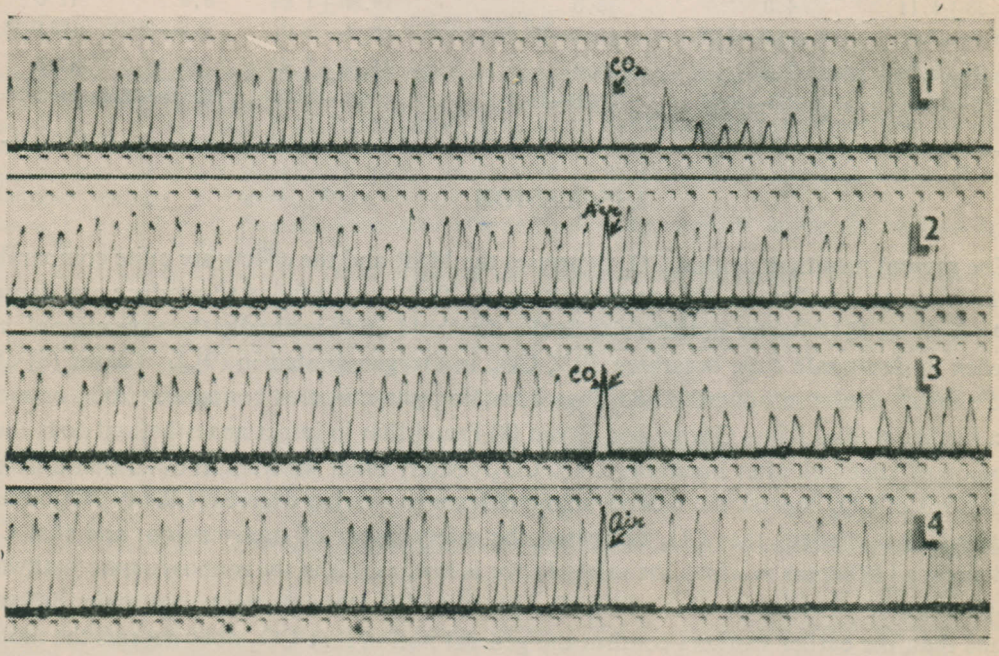

Fig. 4: Shows the oscilloscopic recording of MSR and the effects of CO_2 and air (strips 1 and 2) in intact animal. Strips 3 and 4 show the responses after bilateral vagotomy.

TABLE I: Shows the monosynaptic reflex heights in mm (Mean ±S.D.) and the range in perenthesis & the effect of Co_2 and air or 100% N_2 before & after bilateral vagotomy.

	Before bilateral vagotomy			*After bilateral vagotomy*		
Cat. No.	*Control ±S.D.*	*After CO_2 (peak depression level)*	*After air or N_2 (peak depress ession level)*	*Control ±S.D.*	*After CO_2 (epak depression level)*	*After air or N_2 (peak depression level)*
8	19.45±1.63 (14.1—22.50)	12.2 (12.2—19.5)	18.0 (18.0—22.0)	18.19±1.82 (15.5—22.0)	8.8 (8.8—17.8)	11.2 (11.2--15.5)
9	13.41±1.84 (9.5—19.2)	10.0 (10.0—16)	17.5 (17.5—18.2)	14.31±0.09 (11.5—16.8)	7.0 (7.0—14.8)	10.4 (10.4–16.5)
10	18.76±1.26 (17.0—22.0)	16.2 (16.2—21.0)	17.0 (17.0—23.0)	19.99±0.90 (17.4—22.5)	12.5 (12.5—17.4)	17.5 (17.5-20.5)
11	19.44±1.49 (16.5—23.5)	14.1 (14.1—20.0)	17.0 (17.0—23.0)	19.89±0.93 (16.5—23.5)	8.8 (8.8—19.0)	17.5 (17.5-20.5)
12	18.98±2.27 (12.5—23.5)	7.0 (7.0—20.0)	14.0 (14.0—20.5)	19.50±2.61 (13.8—24.0)	9.5 (9.5—24.0)	16.0 (16.0-26.0)
13	17.30±2.76 (11.0—22)	5.5 (5.5—14.0)	16.0 (16.0—19.0)	20.15±1.55 (16.8—23.0)	12.0 (12.0—22.2)	19.5 (19.5-22.0)
16	18.28±1.11 (14.8—21.0)	4.0 (4.0—21.0)	15.0 (15.0—23.5)	19.86±1.64 (15.2—24.0)	6.0 (6.0—24.0)	12.0 (12.0-24.0)
17	19.68±1.34 (15.5—22.2)	7.0 7.0—20.5)	16.0 (16.0—19.0)	21.86±1.43 (18.0—25.0)	10.0 (10.0—22.2)	17.5 (17.5-25.0)

DISCUSSION

The present results show that CO_2 depresses the MSR and the effects are quite comparable to the PDG depression effects. By contrast nitrogen (100%) or air failed to produce any depression. As early as 1912 Porter noted a rise in the threshold and eventually the extinctionof the flexor reflex in decapitate cats, breathing 50% CO_2 in Oxygen [noted in (9)]. Glazer (6) also obtained similar results in anaesthetised dogs breathing gas mixtures containing 20% or more CO_2. In further studies King, Garrey and Bryan (8) found that in intact anaesthetised dogs there is depression of knee jerk after breathing 2-4% CO_2. Many other workers showed that different concentrations of CO_2 depress the monosynaptic reflexes to varying degrees (1,9,14). Woodbury and Karler (12) suggested that CO_2 decreased the safety factor of transmission, and that CO_2 would depress all synapses in which the safety factor is low, as it is in the monosynaptic pathways in the spinal cord. The result obtained in the present series though conducted under different conditions than the earlier workers confirm the general notion that CO_2 does depress the monosynaptic reflexes. It may be argued that the volume of gases used in the present experiments, being virtually twice the tidal volume in cat, could have by itself stimulated the stretch receptors

f the lung (13). The fact, however, that 60 *ml* of air or N_2 did not being about any change in e monosynaptic reflex, while identical volume of CO_2 (60 *ml*) brought about a significant depres-ion, suggests that the activation of stretch receptors or hypoxia played no part in depressing the nonosynaptic reflexes. This effect was rather CO_2 specific. Further as the animals were atro-inished, it would indicate that bradycardia or hypotension could not be the possible cause of dep-ession of monosynaptic reflexes (3). Since CO_2 continues to depress the monosynaptic reflexes fter vagotomy in contrast to the abolition of PDG induced J-reflex (3), it would indicate that e pathway for CO_2 cannot be meaningfully used to study the J-reflex in as much as the afferent athway for J-reflex is known to be mediated via non-medullated vagal fibres (11). Also, the fact hat CO_2 affects CNS directly at multi-levels and causes the depression of MSR, raised a basic uestion about the use of CO_2 as a specific test sunstance to investigate a particular reflex. The resent studies are, therefore, a pointer in this direction to show the inadequacy of using Co_2 as a method to study a reflex in general, and J-reflex in particular. Granted this limitation, it would ill be of interest to find out the site and nature of pathway involved in Co_2 indiced depression of M.S.R.

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