Pharmacology Biochemistry & Behavior, Vol. 17, pp. 19–23, 1982. Printed in the U.S.A.

Pulmonary Vagal Afferent Stimulants in the Conscious Rat: Opioids and Phenyldiguanide

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Received 10 November 1981

WILLETTE, R. N., P. GATTI, S. B. GERTNER AND H. N. SAPRU. *Pulmonary vagal afferent stimulants in the conscious rat: Opioids and phenyldiguanide.* PHARMAC. BIOCHEM. BEHAV. 17(1) 19–23, 1982.—Phenyldiguanide (PDG, 40 μg/kg), D-ala^2-met^5-enkephalinamide (D-AME, 250 μg/kg) and morphine sulfate (MS, 2 mg/kg) injected into the right atrium (RA) of conscious freely moving rats produced a profound bradycardia and hypotension 1–2 sec subsequent to administration. Concomitant with the cardiovascular effects apnea occurred and lasted approximately 5 sec. Atropine methyl nitrate (2 mg/kg, RA) significantly attenuated the bradycardia and hypotension produced by all three agents. Naloxone blocked only the opioid responses. Coordinated motor activity was impaired following the administration of PDG (40 μg/kg, RA). Fifty percent of the animals receiving PDG failed to remain on a rotor rod for a 2 min period. Only 8 percent of the saline treated group fell off during this period. It was concluded that the cardiovascular, respiratory, and motor effects caused by PDG, in the conscious freely moving rat, were the result of stimulation of pulmonary vagal afferents (J-receptors). The cardiovascular effects of opioids are also believed to arise from the stimulation of J-receptors. However, unlike PDG, these effects are mediated by pulmonary opiate receptors.

Enkephalin Morphine Pulmonary J-receptors Motor activity Vagal afferents Cardiovascular reflex Pulmonary opiate receptors

RECENTLY, a pulmonary opiate receptor reflex has been described [16]. This reflex is initiated by an interaction of opioid agonists with opiate receptors in the lung accessible through the pulmonary circulation. Agonist interaction with pulmonary opiate receptors causes stimulation of vagal afferent type J-receptor fibers. Stimulation of these vagal afferents with Phenyldiguanide (PDG) has been shown to evoke profound alterations in respiratory, cardiovascular and motor functions [8,14]. Respiratory responses include apnea followed by rapid shallow breathing and cardiovascular responses include a dramatic bradycardia with consequent hypotension followed by a slight rise in blood pressure. Somatic responses comprising the J-reflex include inhibition of the crossed extensor reflex [11], depression of gamma efferent activity [10], and depression of evoked alpha-motoneuron activity. In the decerebrate rat, the right atrial administration of opioid peptides cause reflex cardiorespiratory effects identical to those observed with PDG. However, opioid actions are blocked by pretreatment with naloxone, an opiate antagonist, while PDG is not.

Studies of the reflex effects of opioids and PDG have been limited to anesthetized and decerebrate animals. The obvious limitations of these animal preparations complicate the extrapolation of results to the conscious state. For this reason the present study was carried out to determine: (1) the cardiovascular effects of a peripherally acting opioid peptide, PDG, and opiates known to have central effects, (e.g., morphine); and (2) the behavioral and motor effects of pulmonary J-receptor stimulation. D-Ala^2-Met^5-enkephalinamide was the opioid peptide chosen to stimulate pulmonary opiate receptors. This peptide was selected because it is a stable analogue of a naturally occurring enkephalin [15], and it is not believed to cross the blood-brain barrier in significant concentrations when administered intravenously [6].

METHOD

Forty-six male Wistar rats (Royal Hart Farms, Middletown, NY) weighing between 200–300 g were used in this study. Animals were housed in standard animal facilities providing constant temperature and alternating 12 hr light and dark cycles. They were provided with standard laboratory rat chow and tap water ad lib.

Surgical Preparation

On the day of the experiment, rats were initially anesthetized with 3% halothane in oxygen and maintained at

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a concentration of 2%. A small midline incision was made along the ventral surface of the neck to expose the right external jugular vein and the left common carotid artery. Under an operation microscope (Carl Zeiss, Inc., New York NY), a premeasured cannula (PE 50) was inserted into the right external jugular vein and advanced until its tip was in the right atrium. The position of the cannula was always confirmed by postmortem examination. Next, the left common carotid artery was isolated and was also cannulated using PE 50 tubing. This cannula was advanced approximately 2.8 cm so that its tip would lie in or near the aortic arch. Arterial cannulae were filled with heparinized saline (200 U/ml, Sigma Chemical Company, St. Louis, MO) to prevent clotting. The cannulae were then passed under the skin using a large blunted 15 gauge needle. The cannulae were exteriorized at the back of the neck via a small incision. Following this, both the ventral and dorsal incisions were sutured using size 4.0 silk. The cannulae were trimmed and sealed with a piece of 23 gauge music wire (Small Parts, Miami, FL). The animal was then taken off the halothane and allowed to recover in its own plastic cage for at least 2 hours.

Cardiovascular Recordings and Intra-Atrial Injections

At least two hours after surgical preparation, individual rats were placed in Lucite cages (28×28×32 cm). The animal was allowed to acclimate in the cage fifteen minutes before cardiovascular recordings were initiated. At that time, the animal's carotid artery cannula was fitted to a swivel cannula (Instech Laboratories, Model 375/22) connected to a pressure transducer (Statham P23 Db) resting on top of the cage for blood pressure measurements. Heart rate was obtained from a tachograph (Grass, 7P4F) triggered by the blood pressure pulse. Blood pressure and heart rate were recorded on a polygraph recorder (Grass, Model 79D). Control blood pressure and heart rate values were recorded for a minimum period of fifteen minutes to insure stable baseline recordings before drugs were injected. All recordings were made between 1200 and 1600 hr.

Drugs were injected into the right atrium (RA) through the exteriorized right atrial cannula. The cannula was connected to a three way stopcock via a 20 cm length of PE 50 tubing filled with phosphate buffered saline (PBS). The injections were made slowly (10–15 sec) and then subsequently flushed with 0.4 ml of PBS.

Assessment of Coordinated Motor Activity Using the Rotor Rod

A simple apparatus was assembled to test for the production of motor deficits in rats. It consisted of a small adjustable motor whose armature turned a dowel (2.5′×2″). The apparatus was similar to that described by Dunham and Miya [5]. The rheostat was set so that the rod made 5 revolutions per minute. Prior to drug injection, the animals were trained to walk on the rotor rod; training enabled them to remain on it for at least a three minute period. On the average, it took most animals a few minutes to master the rod. After learning to walk on the rod, the animals were then rested for 3–4 minutes. Before being put back on the rod, the animals' exteriorized right atrial cannula was connected to a three way stopcock and injections were made once the animals were on the rod for 30 seconds. All animals tested received both phosphate buffered saline (PBS) and PDG (40 μg/kg) through the atrial cannula in a random order during separate exposures to the rotor rod. Those animals which fell from the rotor rod within 2 minutes following the injections were positive responders while those which did not fall within this time period were negative responders.

Drugs

The following drugs were dissolved in PBS and injected into the right atrium (RA) in a volume of 0.1–0.2 ml to assess their effects on blood pressure, and heart rate: (1) D-Ala^2-Met^5-enkephalinamide (DAME, 250 μg/kg, Boehringer Mannheim Biochemicals, Indianapolis, IN). (2) Phenyldiguanide (PDG 40 μg/kg, Aldrich Chemical Company, Milwaukee, WI). (3) Morphine Sulphate (MS, 2 mg/kg, Merck, Raritan, NJ). PDG was also tested to determine its ability to produce a motor deficit. In some cases animals were pretreated with either an opiate antagonist: naloxone hydrochloride (800 μg/kg, IV, Endo Laboratories, Garden City, NY), or a muscarinic antagonist: atropine methynitrate (2 mg/kg, IV, Sigma Chemical, St. Louis, MO).

Statistical Analysis

All results were expressed as mean±S.E. with $p<0.05$ considered as the level of significance. Quantal responses obtained in the rotor rod experiments were compared using the Chi-Square test [2].

RESULTS

Blood Pressure and Heart Rate

The effects of PDG (40 μg/kg, RA), DAME (250 μg/kg, RA) and MS (2 mg/kg, RA) on the pulsatile carotid arterial blood pressure (BP) and heart rate (HR) were determined in 15 unanesthetized freely moving rats. These animals were divided into 3 groups of 5 animals; with each group receiving only one of the three drugs (PDG, D-AME, or MS). Preliminary experiments in decerebrate rats showed these doses to be equipotent with respect to the duration of apnea produced by their activation of pulmonary vagal afferents. In the present experiments, control mean arterial pressure (MAP) and HR were 114.2±2.5 mm Hg and 382.9±9.6 beats/min, respectively. Within 1–2 sec subsequent to the administration of PDG, DAME, and MS, there was a dramatic bradycardia and hypotension in all animals tested. Typical responses for equipotent doses of PDG (40 μg/kg), DAME (250 μg/kg), and MS (2 mg/kg) are shown in the polygraph tracings of Fig. 1. The bradycardia induced by the right atrial administration of PDG, DAME, and MS was dose related in the ranges of 1.5–40 μg/kg, 2.5–250 μg/kg, and 150–2000 μg/kg, respectively. Threshold dose ranges were: 1.2–1.4 μg/kg (PDG), 2.5–7.0 μg/kg (DAME), and 150–200 μg/kg (MS). These responses are characteristic of pulmonary vagal C fiber stimulation [1]. Stimulation of these nerve fibers is thought to transmit nociceptive or dyspneic sensations (Widdicombe [18]). However, there was no evidence of pain or discomfort, e.g., vocalization and motor activation. On the contrary, the animals remained motionless and appeared sedated for approximately 12 min following PDG and DAME. During this time they responded to prodding and tail pinch. The MS group remained practically motionless for approximately 90 minutes. Micturition was observed in 55% of the animals within 30 sec of the administration PDG, DAME, and MS. It was also noted that the period of apnea coincided with the acute phase of bradycardia, and was followed by a

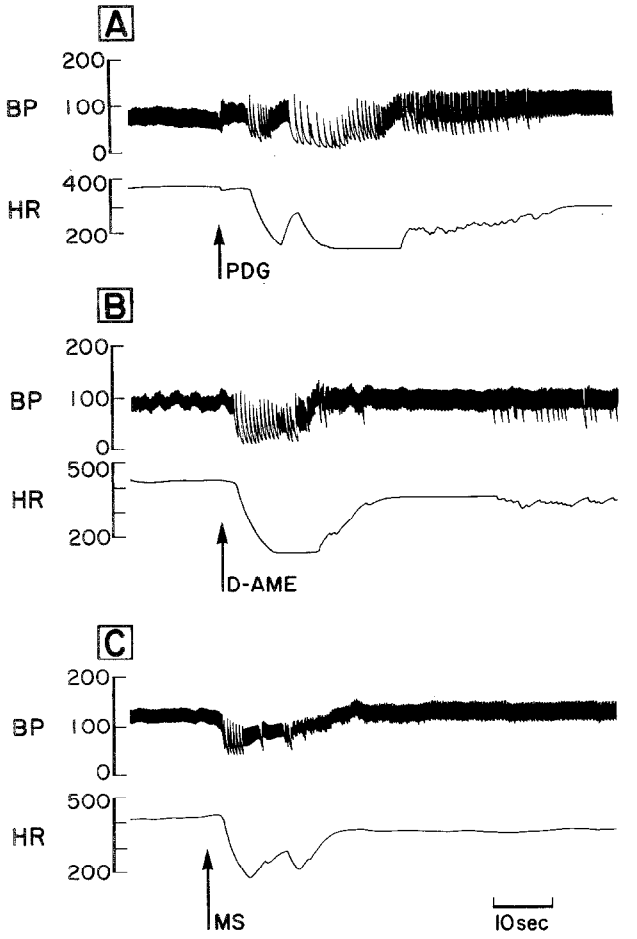

FIG. 1. Polygraph tracings of blood pressure and heart rate responses to: (A) phenyldiguanide (PDG, 40 μg/kg, RA), (B) D-ala^2-met^5-enkephalinamide (D-AME, 250 μg/kg, RA) and (C) Morphine Sulfate (MS, 2 mg/kg, RA). Drugs were injected in equal volumes at the arrows.

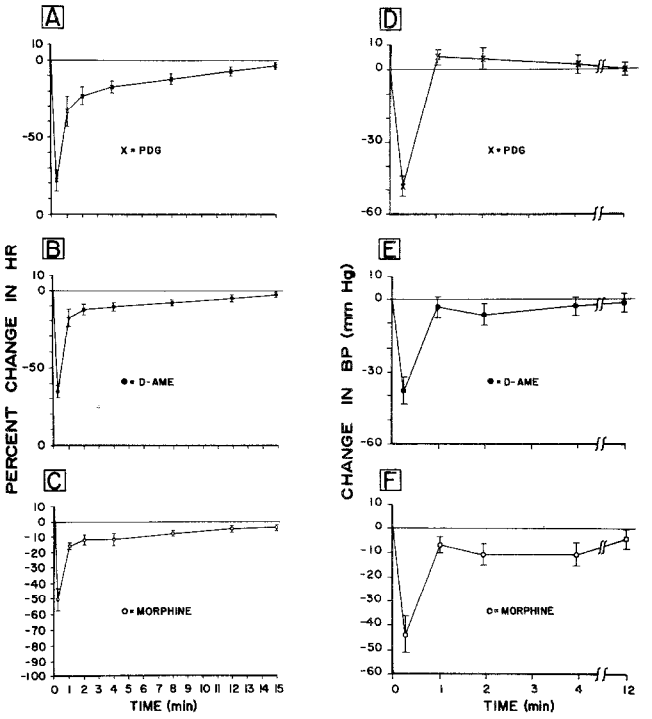

FIG. 2. Magnitude and time course of the blood pressure and heart rate response to PDG (40 μg/kg, RA), D-AME (250 μg/kg, RA) and MS (2 mg/kg, RA), are shown in these graphs. Panels A–C are the mean percentage change in heart rate (HR) obtained for a total of 5 rats for each drug (1 dose/rat). Panels D/F are the mean arterial blood pressure (BP) responses for each group of animals used to determine the HR responses. All drugs were administered in equal volumes at time 0.

change in respiratory efforts. Only MS caused a complete tachphylaxis following the recovery of BP and HR; when subsequent doses of MS were administered 30 minutes after an initial dose no changes in HR and BP were observed. After 3 hr the animals responded partially to morphine.

The magnitude and time course of the HR and BP responses are depicted graphically in Fig. 2. The maximal fall in HR and BP occurred within 15 sec of the administration of PDG, DAME, and MS. This amounted to a reduction in HR of 291±26 bpm (78±7%) for PDG, 254×15 bpm (65±4%) for DAME, and 196±27 bpm (50±7%) for MS (Fig. 2a–c).The bradycardia for all three agents persisted for approximately 15 min; at which time the rate had returned to predrug levels. Frequently, arrhythmias were apparent during the first 2 min after PDG, DAME, and MS administration. Injections of 0.6 ml of vehicle (PBS, pH 7.4) into the right atrium caused only a slight (28±5 bpm) reduction in HR for the duration of the injection.

The mean arterial pressure (MAP) response, to the right atrial administration of PDG, DAME, and MS, consisted of hypotension which paralleled the initial phase of the HR response (bradycardia). Within the first 15 sec, the changes in MAP (mm Hg) were −48±5, −38±7 and −44±7 mmHg, for PDG, DAME, and MS respectively (Fig. 2d–e). Unlike the HR response, MAP returned to predrug levels in 1 min. During this period the pulse pressure increased.

Pretreatment of 9 animals with atropine methyl nitrate (2 mg/kg, RA) caused HR to increase 39±4 bpm. In this pretreated group, the right atrial administration of PDG, DAME, and MS did not produce bradycardia (Fig. 3). Although the fall in MAP in response to PDG, DAME, and MS was greatly reduced in the atropinized group, a transient hypotension (−18±4, −12±2 and −7±3 mm Hg respectively) still persisted in most animals. Following the fall in BP a rise was often observed.

Nine animals were pretreated with the opiate antagonist naloxone HCl (10–800 μg/kg, RA). Naloxone itself had no noticeable effects on HR and BP, but it did completely block the responses caused by MS. Naloxone was effective in abolishing these responses at doses as low as 10 μg/kg (RA). On the other hand, the reflex cardiovascular responses induced by PDG were unaltered by the antagonist. These experiments support the view than opiate receptors in the lung mediate a pulmonary depressor reflex (Fig. 4).

Coordinated Motor Activity

The results of a simple test for coordinated motor activity (described in the Method Section) are shown in Table 1). Fifty percent of the animals receiving PDG (40 μg/kg, RA)

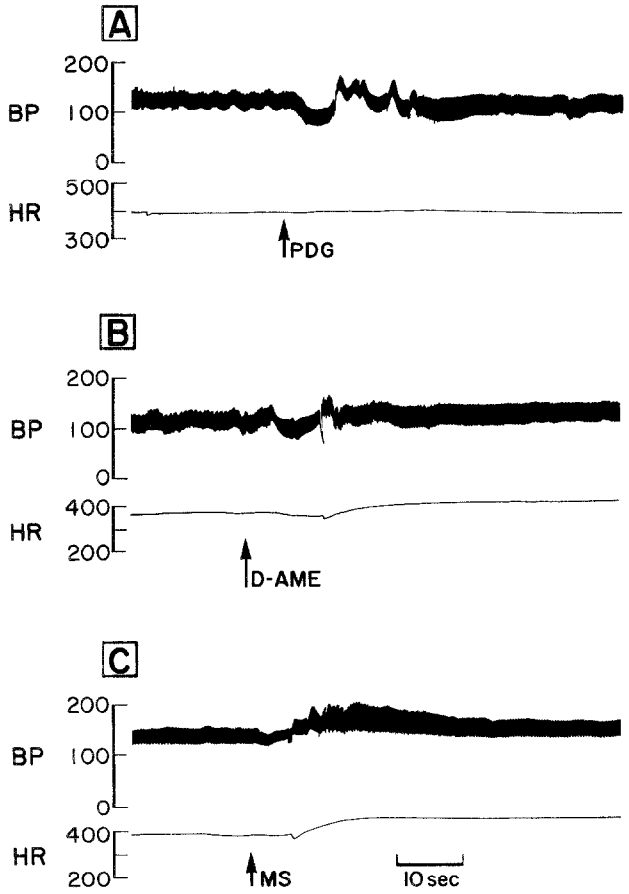

FIG. 3. Polygraph tracings of blood pressure and heart rate responses to: (A) PDG (40 μg/kg, RA) (B) D-AME (250 μg/kg, RA) and (C) MS, (2 mg/kg, RA) five minutes after methylatropine was administered (2 mg/kg, RA). Drugs were injected in equal volumes at the arrows.

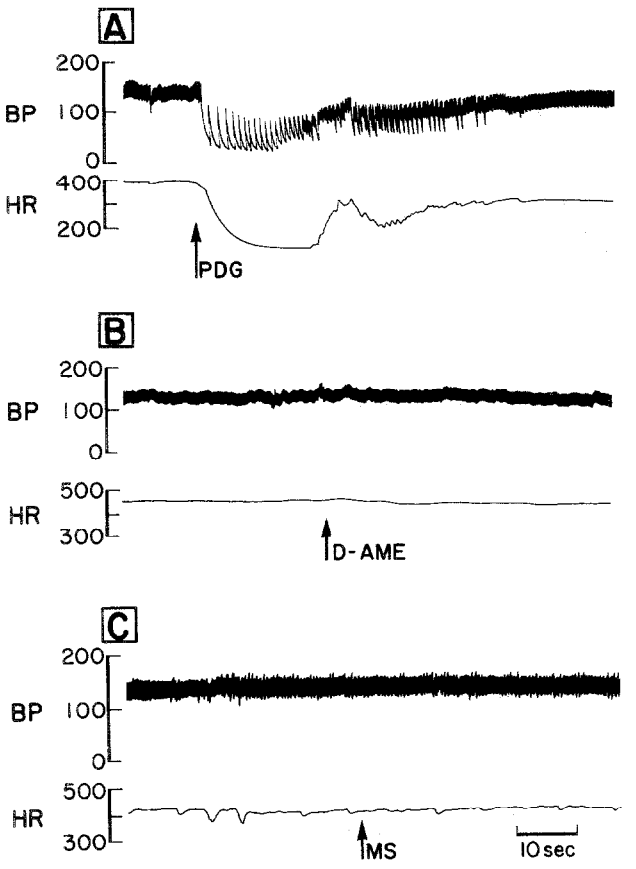

FIG. 4. Polygraph tracings of blood pressure and heart rate response to: (A) PDG (40 μg/kg, RA), (B) D-AME (250 μg/kg, RA) and (C) MS (2 mg/kg, RA), five minutes after naloxone was administered (800 μg/kg, RA). Note that the cardiovascular responses to PDG were not blocked by naloxone. Drugs were injected in equal volumes at the arrows.

responded positively, i.e., they fell from the rotor rod within 2 minutes. On the other hand, only 8% of the animals receiving an equal volume of phosphate buffered saline (RA) were positive responders. MS and D-AME were not administered in these experiments because of the possibility of complicating central and spinal actions [19].

DISCUSSION

In the present study, phenyldiguanide (PDG) was employed for the stimulation of afferent vagal ending, i.e., pulmonary J-receptors, in conscious freely moving rats. Within 1–2 sec subsequent to the administration of PDG (40 μg/kg) into the right atrium (RA), a powerful depressor reflex was evoked. The reflex effects were similar to those attributed to pulmonary J-receptor activation in the anesthetized cat [3, 10, 14] and dog [1].

Recently, a pulmonary opiate receptor reflex has been described by Sapru *et al.* [16]. In decerebrate rats, the reflex cardiorespiratory actions of opioids, as well as their effects on pulmonary vagal afferents, were analogous to those elicited by PDG. In the present study, the cardiovascular responses in the conscious rat obtained for morphine sulfate (MS, 2 mg/kg, RA) and D-ala^2-met^5-enkephalinamide (DAME, 250 μg/kg, RA) were also virtually identical to those obtained with PDG (40 μg/kg, RA).

By observing the respiration before, during, and after the administration of PDG, MS, and DAME, it was apparent

TABLE 1

	Rotor Rod		
	(+)	(−)	Total
Saline	1	11	12
PDG*	6	6	12

Twelve rats were acclimated to a rotor rod (5 rpm) and were able to remain on the rod for at least 3 min. Following each 3 min exposure the animals were rested for 3 min. Each animal received both saline and PDG (40 μg/kg, RA) on separate rotor rod exposures. Negative responders were able to walk the rod for at least 2 min following the administration of PDG or an equal volume of its vehicle (saline).

*$p<0.05$.

that there were substantial changes. Immediately after the administration of the compounds, there was apnea, followed by what appeared to be labored rapid breathing. In one congested animal the subsequent injection of PDG induced a distressing situation characterized by strenuous respiratory efforts and pallor.

The arterial blood pressure (BP) responses following the right atrial administration of PDG, D-AME, and MS, were similar. There was a fall in BP which was primarily the result of a reduction in the cardiac output caused by vagally mediated reflex bradycardia. However, an initial fall in BP was observed in most animals following blockade of the bradycardia with atropine methyl nitrate (2 mg/kg, IV). This initial fall in BP was often followed by an increase in BP; similar to that reported by Schaz *et al.* [17] for intravenously administered opioids. It is probable, that the overall BP response, in atropinized animals, represents the sum of opposing actions of increasing pCO2 (during apnea) and a direct sympathoinhibitory action of activated pulmonary vagal afferents.

Vagally mediated bradycardia, following the intravenous administration of morphine-like compounds, has been well documented [12]. These investigators have concluded that this effect is the result of an interaction with opioid receptors in the central nervous system. Indeed, perfusion of the IV ventricle with morphine-like compounds [7] and microinjections of DAME into the nucleus ambiguus causes vagally mediated bradycardia [13]. It is doubtful, however, that bradycardia elicited by injections of opioids into the right atrium is mediated centrally. This view is supported in the present study by the brief time to onset of action and ability of the opioids to mimic PDG's effects. The reflex bradycardia caused by PDG, DAME, and MS was particularly powerful and intravenous doses of atropine methylnitrate as high as 1 mg/kg did not completely block the fall in heart rate.

Stimulation of pulmonary vagal afferents (J-receptors) has been shown to inhibit somatic functions (the J-reflex). Monosynaptic [4] and polysynaptic reflexes as well as spontaneous motor activity, has been shown to be inhibited by PDG in a manner dependent upon intact vagi and basal ganglia [9,11]. For review, see Ginzel [8]. In this report, the rotor rod apparatus was used to test for impairment of coordinated motor activity. Animals receiving PDG (40 μg/kg, RA) were less apt (50%), than the saline group (92%) to remain on the rod 2 min after injections. This can be interpreted as preliminary evidence for the impairment of coordinated motor activity by stimulation of J-receptors. Opiates were not tested in this experiment because of the possible complication of central depressant actions. However, evidence indicates that the same J-receptors stimulated by PDG are also stimulated by opioids [16]. This may imply complications in the interpretation of analgesic tests dependent upon a muscular act as an end point; especially when the opioid being tested is administered intravenously, a route of administration sure to rapidly deliver a large percentage of the dose to the pulmonary circulation.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. George A. Condouris for the generous contribution of morphine sulfate and Ms. Pam Koontz for her help in preparing this manuscript. This work was supported by Grant HL 24347 from the National Institute of Health awarded to Dr. H. N. Sapru.

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