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Blockade by Naltrexone of Analgesia Produced by Stimulation of the Dorsal Raphe Nucleus[1]

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SWAJKOSKI, A. R., D. J. MAYER AND J. H. JOHNSON. *Blockade by naltrexone of analgesia produced by stimulation of the dorsal raphe nucleus.* PHARMAC. BIOCHEM. BEHAV. 15(3) 419–423, 1981.—Naloxone blockade of stimulation-produced analgesia in the rat is partial and variable. In the present study the effectiveness of the long-acting narcotic antagonist naltrexone is examined. Bipolar stainless steel electrodes were implanted in the dorsal raphe nucleus or ventral periaqueductal gray matter of male rats. Analgesia produced by electrical stimulation was tested by the tail flick method before and twenty min following the administration of saline or naltrexone. Saline administered IP failed to alter the analgesic response. Following naltrexone the degree of analgesia was reduced by a mean of 79% for IV injection (3.7 mg/kg) and by means of 26%, 52%, 81% and 83% for IP administration of 0.3, 1.0, 3.0 and 10 mg/kg, respectively. These results confirm the participation of opiate mechanisms in stimulation-produced analgesia, and indicate that, under certain circumstances, only opiate mechanisms are involved.

Naltrexone Dorsal raphe nucleus Analgesia blockade Opiate mechanisms

FOCAL electrical stimulation of the midbrain central gray matter, including the dorsal raphe nucleus (DRN) elicits potent behavioral analgesia in animal models [10,15] as well as in man [1]. Lesion studies and microinjection findings have illustrated functional similarities between SPA and opioid-induced analgesia [8,19]. The blockade of SPA [1,2] and opioid analgesia [19] by the narcotic antagonist naloxone, and the development of cross tolerance between SPA and morphine [14] offer additional evidence concerning respective modes of action and similarities between SPA and narcotic analgesia. However, naloxone only partially inhibits SPA in experimental animal models [2]. The purpose of the present study was to investigate the effect of the clinically long acting narcotic antagonist naltrexone on SPA in the rat.

METHOD

Adult male Sprague-Dawley rats were used in this study. Animals were housed in central facilities utilizing a 14 hr light 10 hr dark cycle. Food (Charles River RH3000) and water were provided ad lib. All rats were anesthetized with Brevital and implanted stereotaxically with electrodes aimed at the DRN (Pellegrino, Pellegrino and Cushman [16]: P0.1, L0.0, V-1.3). Electrode contacts were fastened to a rack and panel connector secured to each animal's skull with dental acrylic. Each electrode consisted of a twisted pair of insulated stainless steel wires having a tip diameter of 0.006 in. Following a 7 day recovery period, each rat was habituated to a fiberglass restrainer for 45 min daily over a 14-day span. The restrainer was utilized for assessment of SPA by a modified version of the tail flick test [6]. Pre-drug latency to removal of the tail from the noxious heat stimulus was determined in 7–9 trials at 2-min intervals. Mean baseline latency was calculated from trials 2–4. Subsequent trials were immediately preceded by a 20-sec application of electrical stimulation, beginning at 0.5 mA (20 Hz, 50 μsec pulse pairs separated by 100 μsec). If the tail flick latency was not increased by at least 1 sec (approximately 20% degree of analgesia) following the first stimulation, the stimulus current was increased in subsequent trials by increments of 0.5 mA up to a maximum of 1.5 mA. Mean test latency was calculated from 3 trials using the same stimulus current. The treatment was administered immediately following the pre-drug testing. Post-drug testing consisted of 4 baseline and 3 stimulation trials at the same final current used before treatment. Mean baseline and test latencies were calculated from 3 trials each, as before. Five groups of male rats were tested before and after IP injection of either heparinized saline or

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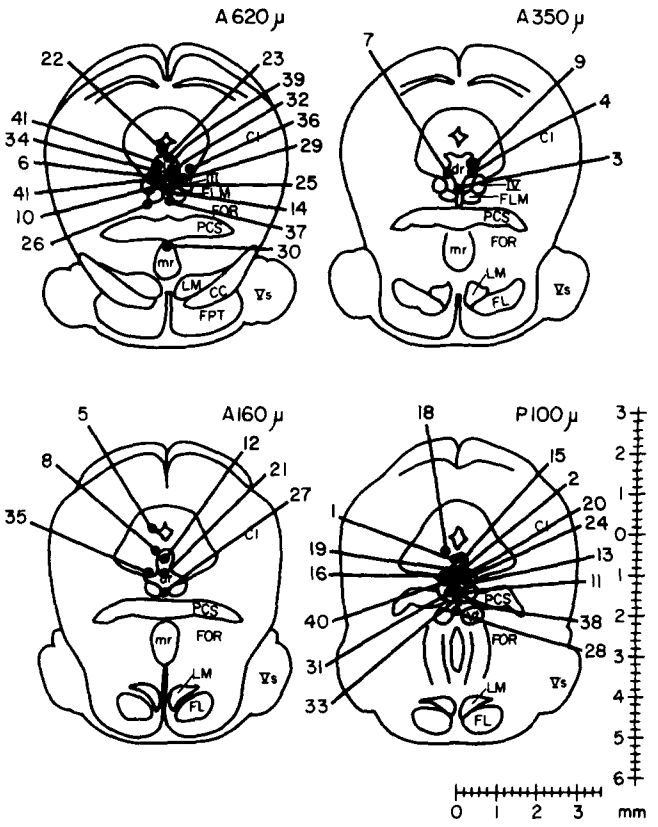

FIG. 1. The approximate location of each stimulating electrode is shown on frontal sections modified from the atlas of König and Klippel [9]. The anterior-posterior level is indicated above each section. Each dot on the map is identified by the Rat no. corresponding to the data in Table 1. Rats 1–9=Saline Treatment; 10–18=Naltrexone, 0.3 mg/kg; 19–26=Naltrexone, 1.0 mg/kg; 27–33=Naltrexone, 3 mg/kg; 34–41=Naltrexone, 10 mg/kg. Abbreviations: CC=crus cerebri; CI=inferior colliculus; dr=dorsal raphe nucleus; FL=longitudinal fasciculus; FLM=medial longitudinal fasciculus; FOR=reticular formation; FPT=transverse pontine fibers; LM=medial lemniscus; mr=median raphe nucleus; PCS=superior cerebellar peduncle; vt=ventral tegmental nucleus; III=oculomotor nucleus; IV=trochlear nucleus; Vs=sensory root of trigeminal nerve.

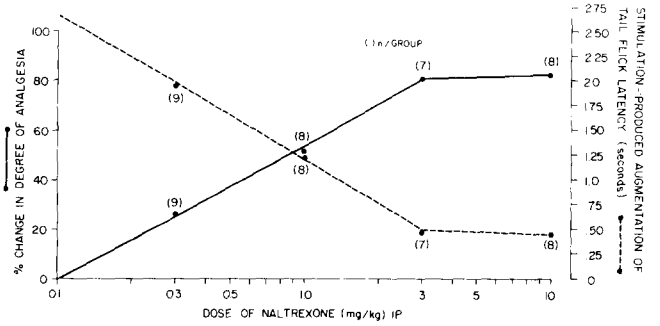

FIG. 2. Dose response relationship for antagonism of stimulus produced analgesia by naltrexone administered IP. The solid line represents the decrease in degree of analgesia for each rat expressed as a percent of the initial value (left axis). Each point represents the mean value for the group. The size of each group is indicated in parentheses adjacent to the point. Analysis by the Litchfield-Wilcoxin method [11] indicated a correlation coefficient of 0.957 and an ED-50 of 0.9mg/kg. The broken line represents the post-naltrexone stimulation-induced augmentation in the tail flick latency (TL-BL) in seconds (right axis). The correlation coefficient for the segment including 0.1–3 mg/kg is 0.9995.

naltrexone HCl (0.3–10 mg/kg) in saline. An additional group of male rats received right atrial cannulae via an external jugular approach 4 days prior to the analgesia testing. The indwelling cannulae were used to infuse naltrexone HCl (3.7 mg/kg) during testing for analgesia. After analgesia testing, animals were sacrificed by barbiturate overdose, and an anodal direct current was passed through each stimulating electrode to deposit iron into tissue surrounding the electrode. Animals were then perfused with potassium ferriferrocyanide solution followed by 10% formalin solution. Electrode locations were verified by comparing 50 μ frozen sections stained with cresyl violet to the atlas [9].

RESULTS

The majority of electrodes were located either in the dorsal raphe nucleus or in the ventral aspects of the central gray matter (Fig. 1). Stimulation significantly increased tail flick latency in all treatment groups. The effects of IP injection of 0.9% saline in 9 male rats are listed in Table 1. No significant difference was observed between mean preinjection (2.83 sec; 78% degree of analgesia: DA) and mean postinjection (2.83 sec; 81% DA) stimulation-induced augmentation in tail flick latency.

Naltrexone significantly reduced the effect of stimulation on the tail flick latency (TL1-BL1 vs TL2-BL2 Student's t: $p<0.003$) at the three higher dosages administered IP (Table 1). Similarly, following IV injections of naltrexone (3.7 mg/kg) the stimulation-induced increase in latency of 3.95 sec (96% DA) was reduced to 0.7 sec (Student's t: $p<0.0001$). No consistent effects of naltrexone on baseline latencies were observed, although those values obtained following the 1 mg/kg dosage were significantly increased (Student's t: $p<0.05$) compared to pre-naltrexone values.

Figure 2 represents the log dose-response relationship between naltrexone administered IP and both the mean augmentation of tail flick latency following stimulation, as well as the percent change in DA in the 4 groups of male rats tested. The maximal response to naltrexone was obtained at 3.0 mg/kg and 10.0 mg/kg, and potency diminished with dosage below 3.0 mg/kg (r=0.9995 for the submaximal response). Analysis of the dose response curve for percent change in DA by the Litchfield-Wilcoxin method [11] indicated an ED-50 of 0.9 mg/kg.

DISCUSSION

The results of the present experiments in which naltrexone antagonized SPA stand in contrast to the ineffective [20], marginal [17] or partial [2] antagonism observed following treatment with naloxone. A much more consistent and dramatic reduction in tail flick latency increment (from 3.03 to 0.48 sec) and in degree of analgesia (77% to 16%) was found 20 min following naltrexone administration (3 mg/kg) than was observed 20 min after naloxone in doses as high as

TABLE 1

EFFECTS OF SALINE OR NALTREXONE (IP) ON SPA IN MALE RATS

		Pre-Naltrexone			Post-Naltrexone		
	Rat No.	BL*	TL†	DA††	BL*	TL†	DA††
Group 1							
1.0 ml/kg	1	3.27	4.60	28	3.63	4.93	30
Saline	2	3.63	5.43	41	3.70	5.93	52
	3	3.83	8.00	100	5.87	8.00	100
	4	4.67	7.53	86	3.93	7.77	94
	5	4.53	8.00	100	4.13	8.00	100
	6	4.83	7.40	81	4.43	8.00	100
	7	4.30	8.00	100	4.13	7.87	97
	8	4.53	6.70	62	4.80	6.50	53
	9	4.63	8.00	100	4.90	8.00	100
	Mean	4.25	7.07§	77.7	4.39	7.22§	80.6
	±SEM	0.18	0.42	9.2	0.24	0.38	9.2
Group 2							
0.3 mg/kg							
Naltrexone	10	4.40	8.00	100	4.20	6.27	54
	11	3.33	8.00	100	3.90	7.83	96
	12	4.97	7.63	88	4.93	5.23	10
	13	4.17	8.00	100	4.27	8.00	100
	14	3.70	6.97	76	4.07	5.80	44
	15	4.27	5.17	24	4.60	5.40	24
	16	3.87	7.27	82	4.53	6.97	70
	17	3.93	5.80	46	4.73	5.63	28
	18	3.93	6.23	57	5.23	6.83	58
	Mean	4.06	7.01§	74.7	4.50	6.44§	53.7
	±SEM	0.15	0.35	9.0	0.14	0.34	10.4
Group 3							
1.0 mg/kg							
Naltrexone	19	4.93	8.00	100	4.80	7.07	71
	20	3.63	5.53	44	4.50	5.53	30
	21	3.47	6.73	72	4.30	5.53	33
	22	4.43	6.43	56	4.47	5.47	28
	23	3.67	5.30	38	4.47	5.17	20
	24	3.73	6.33	61	4.47	5.03	16
	25	4.03	8.00	100	3.97	6.70	68
	26	4.23	7.23	80	4.57	4.77	6
	Mean	4.02	6.70§	68.7	4.44**	5.66§#	33.9
	±SEM	0.17	0.36	8.4	0.08	0.29	8.3
Group 4							
3 mg/kg							
Naltrexone	27	4.73	6.90	66	5.50	5.03	0
	28	3.73	7.77	95	5.13	5.47	12
	29	4.70	7.23	77	5.13	5.27	5
	30	2.73	4.67	37	2.60	2.93	6
	31	4.47	7.47	85	5.43	6.17	29
	32	3.37	6.97	78	3.93	5.10	29
	33	4.03	8.00	100	4.97	6.10	37
	Mean	3.97	7.00§	76.7	4.67	5.15#	14.1
	±SEM	0.28	0.42	7.9	0.41	0.40	7.2

TABLE 1
continued

Group 5							
10 mg/kg							
Naltrexone	34	5.27	8.00	100	5.47	5.50	1
	35	3.20	5.90	56	3.43	3.67	5
	36	3.53	7.93	98	3.63	4.37	17
	37	4.63	7.13	74	5.20	5.27	2
	38	3.67	6.50	65	4.93	5.60	22
	39	3.97	7.50	88	4.23	4.37	4
	40	4.13	7.03	75	4.10	4.87	20
	41	4.47	7.07	74	3.80	4.77	23
	Mean	4.11	7.13§	78.8	4.35	4.80#	11.7
	±SEM	0.24	0.25	5.4	0.27	0.23	3.3

*Baseline (prestimulation) tail flick latency.
†Poststimulation tail flick latency.
††Degree of analgesia $= \frac{TL-BL}{8-BL} \times 100$.
§Greater than corresponding BL $p<0.05$ (t-test).
¶Less than corresponding pre-naltrexone DA $p<0.01$ (t-test).
#Less than corresponding pre-naltrexone TL $p<0.05$ (t-test).
**Greater than corresponding pre-naltrexone BL $p<0.05$ (t-test).

4 mg/kg (100% to 62% and 58% to 36%) [2]. This may indicate that naltrexone exerts a more potent inhibitory effect on SPA, or may reflect the shorter duration of action of naloxone. Naltrexone has been reported to be approximately three times as potent as naloxone in binding to opiate receptors [5] and in precipitating withdrawal [12], although naloxone enters the brain more rapidly [7]. An additional difference between these two narcotic antagonists is seen in their effects on baseline latencies in the tail flick test. Naloxone has been reported to lower this latency [3]. No consistent, dose-related effect of naltrexone on baseline latencies was observed in the present experiments, and the sole significant effect was a slight increase in the baseline latency observed following the administration of 1 mg/kg. This is consistent with reports of a mild agonist effect of naltrexone in the rat [4], and indicates that the dramatic antagonism of SPA cannot be accounted for by naltrexone-induced hyperalgesia.

An alternative explanation for the greater efficacy of naltrexone in antagonizing analgesia involves differences in sites of stimulation, in view of the greater susceptibility to naloxone of analgesia produced by stimulation of ventral as opposed to dorsal regions of the periaqueductal gray matter [18]. In the present study, stimulating electrodes were located predominantly in the ventral PAG or in the DRN whereas the study of Akil *et al.* [2] included more dorsally situated sites.

It is apparent from these data that opiate mechanisms are crucial to the elicitation of analgesia by electrical stimulation in the region of DRN and the adjacent PAG. A clear dose-response relationship with a high correlation coefficient has been demonstrated for naltrexone. Such a relationship was not observed for naloxone [2,17]. The present results indicate that under certain conditions a nearly complete reversal of SPA can be attained using a narcotic antagonist. Moreover both the significant SPA and its consistent antagonism by naltrexone were obtained in tail flick tests performed after the termination of stimulation. These observations indicate that any differences in responses obtained during, versus following, stimulation [18] are not crucial to the demonstration of opiate receptor participation. On the other hand, persistence of analgesic responses in the face of challenge by naloxone [2, 17, 20] can be taken as evidence for the existence of additional pain-inhibitory mechanisms which do not depend on opiate systems. Such a conclusion is consistent with those derived from other lines of evidence [13]. Although a number of variables examined in our experiments as well as those of others may be critical in determining the particular neural system activated (e.g. electrode site, stimulation intensity or duration, etc.) no such variable has yet been implicated with any certainty. The clarification of this point should provide valuable information about the nature of pain modulatory systems.

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