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Potentiation of Cold-Water Swim Analgesia and Hypothermia by Clonidine

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BODNAR, R. J., K. P. MERRIGAN AND E. SPERBER. *Potentiation of cold-water swim analgesia and hypothermia by clonidine*. PHARMACOL BIOCHEM BEHAV **19**(3) 447–451, 1983.—The analgesia induced by acute exposure to cold-water swims (CWS) covaries with levels of brain norepinephrine and is reduced by lesions placed in the locus coeruleus. In assessing whether alpha-noradrenergic receptor mechanisms mediated CWS analgesia, the first experiment found that clonidine pretreatment (500, 1000 μg/kg) elevated jump thresholds 60 min following injection. While clonidine (1000 μg/kg) paired with a 2°C CWS potentiated CWS analgesia in a synergistic manner, additivity of analgesic effects was observed following pairing of clonidine (500 μg/kg) with a 2°C CWS and pairing of clonidine (500 and 1000 μg/kg) with a 15°C CWS. The second experiment showed that clonidine (500 μg/kg) paired with a 2°C CWS enhanced CWS analgesia on the tail-flick test. The third experiment indicated that while clonidine (500 and 1000 μg/kg) or CWS (2°C) each produced hypothermia, pairing of these clonidine doses with CWS enhanced CWS hypothermia. These data are discussed in terms of the possible modulatory role that norepinephrine, and particularly its alpha-noradrenergic receptor subclass, plays in the full expression of CWS analgesia and hypothermia.

Clonidine Cold-water swims Pain Analgesia Rats

IN assessing the physiological and neurochemical substrates of the analgesic response following cold-water swims (CWS: [5]), the catecholamines, dopamine and norepinephrine have been evaluated. While the dopamine receptor stimulant apomorphine reduces CWS analgesia [4], the dopamine receptor blocker chlorpromazine potentiates this analgesic effect [8]. In general, an inverse relationship has been observed for norepinephrine. While stimulation of the nucleus locus coeruleus produces analgesia [26], lesions placed in the locus coeruleus reduce CWS analgesia [10]. Furthermore, while acute exposure to CWS reduces brain norepinephrine levels [28, 29, 31] and produces analgesia [5], chronic exposure to CWS results in adaptation of both effects [6,31]. Noradrenergic effects upon some forms of stress-induced analgesia appear to be mediated through the alpha-noradrenergic receptor since yohimbine reduces the autoanalgesia induced by conditioning to foot shock [12].

Clonidine, an alpha-noradrenergic receptor agonist [1,18] produces analgesia following systemic administration on the vocalization, tail-flick, writhing and yeast-paw tests [15,19]. Clonidine interacts with opioid systems in that it suppresses withdrawal signs which were elicited by naloxone [27]. Yet its analgesic effects are unaffected by opiate receptor antagonism [15]. Repeated clonidine injections result in analgesic tolerance and exhibit cross-tolerance with the autoanalgesic response induced by conditioning to foot shock [11].

To determine whether noradrenergic receptor mechanisms are involved in the mediation of CWS analgesia, the first experiment investigated whether pairing of various (125, 500, 1000 μg/kg) clonidine doses with CWS at bath temperatures of 2°C and 15°C would potentiate jump thresholds to a greater degree than exposure to each manipulation alone. The second experiment examined whether any observed interaction between clonidine and CWS on the jump test would also occur on the tail-flick test. The third experiment evaluated whether any observed interaction in analgesic responses was paralleled by an interaction between hypothermic effects induced by clonidine and CWS.

EXPERIMENT 1

METHOD

The jump thresholds of male albino Sprague-Dawley rats (300–450 g) were determined according to an ascending method of limits procedure [14]. Electric shocks were delivered by a 60 Hz constant current shock generator and a grid scrambler through a 30 cm by 24 cm floor composed of 16 grids. The jump threshold was defined in mA as the lowest of two consecutive intensities that elicited simultaneous withdrawal of both hindpaws from the grids. Each trial began with the animal receiving a 300 msec foot shock at a current intensity of 0.1 mA. Subsequent shocks occurred at 10 sec intervals and were increased in equal 0.05 mA steps until the nociceptive threshold was determined. After each trial, the current intensity was reset to 0.1 mA for the next trial until 6 trials were completed.

To determine the analgesic effects of clonidine on jump thresholds, 24 rats, matched on the basis of baseline jump thresholds, were subdivided into four groups of six rats. Each group received an intraperitoneal injection of clonidine at one of the following doses: 0, 125, 500, 1000 μg clonidine HCl (Catapres)/ml normal saline/kg body weight. Jump thresholds were determined 60 min thereafter. The experimenters conducting the tests were unaware of the injection condition.

TABLE 1

CLONIDINE AND JUMP THRESHOLDS (mA)

		Clonidine Dose (μg/kg)			
		0	125	500	1000
Baseline	mean	0.410	0.427	0.421	0.429
	SEM	0.011	0.024	0.020	0.008
Injection	mean	0.488	0.442	0.631†*	0.754†*
	SEM	0.018	0.045	0.48	0.059

Significant difference (Dunnett Comparisons, $p<0.05$) from baseline (†) or from vehicle condition (*).

To determine the effects of clonidine upon CWS-induced increases in jump thresholds, 48 rats, matched on the basis of baseline jump thresholds, were subdivided into eight groups of six rats. The first four groups received clonidine at doses of either 0, 125, 500 or 1000 μg/kg 30 min before a 3.5 min swim in a 2°C temperature bath. The second four groups received the same clonidine doses 30 min prior to a 3.5 min swim in a 15°C temperature bath. Jump thresholds of all groups were determined 30 min after the swims. The experimenters conducting the tests were unaware of the injection condition.

RESULTS

Clonidine and Jump Thresholds

Significant differences were observed between conditions, $F(1,20)=59.60$, $p<0.01$, and for the interaction between groups and conditions, $F(3,20)=11.40$, $p<0.01$. Table 1 shows that the 500 and 1000 μg/kg, but not the 0 or 125 μg/kg clonidine doses significantly increased jump thresholds above baseline levels. Furthermore, jump thresholds following the 500 and 1000 μg/kg, but not the 125 μg/kg clonidine doses were significantly higher than those following the vehicle injection. As expected, the baseline thresholds failed to differ from each other.

Clonidine and 2°C CWS Analgesia

Significant differences were observed between conditions, $F(1,20)=33.74$, $p<0.01$. Table 2 shows that all CWS conditions significantly increased jump thresholds above baseline levels. Furthermore, the analgesia induced by pairing the 1000 μg/kg clonidine dose with CWS was significantly greater than the analgesia induced by the vehicle-CWS pairing. In contrast, the two lower clonidine doses failed to significantly potentiate CWS analgesia. As expected, the baseline jump thresholds failed to differ from each other.

Clonidine and 15°C CWS Analgesia

Significant differences were observed between conditions, $F(1,20)=10.68$, $p<0.01$. Table 2 shows that the 15°C swim significantly increased jump thresholds above baseline levels when paired with the 500 and 1000 μg/kg clonidine doses, but not when paired with the 0 or 125 μg/kg clonidine doses. However, all clonidine-swim pairings failed to significantly potentiate jump thresholds above the vehicle-swim pairing. As expected, the baseline jump thresholds failed to differ from each other.

TABLE 2

ALTERATIONS IN JUMP THRESHOLDS (mA) FOLLOWING CLONIDINE AND COLD-WATER SWIMS

		Clonidine Dose (μg/kg)			
		0	125	500	1000
		Cold-Water Swims: 2°C			
Baseline	mean	0.411	0.410	0.399	0.401
	SEM	0.046	0.027	0.042	0.036
Swim	mean	0.645†	0.686†	0.827†	1.077†
	SEM	0.050	0.041	0.150	0.180
		Cold-Water Swims: 15°C			
Baseline	mean	0.406	0.413	0.410	0.416
	SEM	0.033	0.025	0.012	0.018
Swim	mean	0.524†	0.549†	0.697†	0.603†
	SEM	0.049	0.033	0.154	0.130

Significant difference (Dunnett Comparison, $p<0.05$) from baseline (†).

TABLE 3

ALTERATION IN TAIL-FLICK LATENCIES (SEC) FOLLOWING CLONIDINE AND COLD-WATER SWIMS

		Tail-Flick Latencies (sec)			
			Post-Injection (min)		
			60	90	120
			Post-Swim (min)		
Condition		PRE	30	60	90
Clonidine	mean	2.80	3.09	3.33	3.55
(500 μg/kg)	SEM	0.08	0.25	0.28	0.15
Cold-Water Swim	mean	2.06	5.19*	4.52*	4.69*
	SEM	0.11	0.44	0.32	0.26
Clonidine +	mean	2.21	5.95*†	5.20*†	5.08*
Cold-Water Swim	SEM	0.12	0.86	1.00	1.00

Significant difference (Dunnett Comparison, $p<0.05$) from PRE (*) or Cold-Water Swims (+).

Clonidine and CWS Interactions

In assessing the relationship between the two analgesic processes, the two higher clonidine doses produced significant analgesia increasing jump thresholds by 0.21 mA (50%) and 0.325 mA (76%) respectively 60 min after injection. While the 15°C CWS increased jump thresholds by 0.118 mA (29%) when paired with a vehicle injection, the two higher clonidine doses paired with this swim condition increased thresholds by 0.287 mA (70%) and 0.187 mA (45%) respectively, indicating that the increases elicited by these pairings were at best additive. While the 2°C CWS increased jump thresholds by 0.234 mA (57%) when paired with a vehicle injection, this condition was potentiated significantly by 0.676 mA (169%) when paired with the 1000 μg/kg clonidine dose. The latter facilitation appears to be greater than the mere addition of the two analgesic effects. Yet since the

TABLE 4

ALTERATIONS IN CORE BODY TEMPERATURE (°C) FOLLOWING CLONIDINE AND COLD-WATER SWIMS (CWS)

Clonidine Dose (μg/kg)	Condition		Core Body Temperature (°C) PRE	Post-Swim (min) 30	60	90
500	No Swim	mean	36.5	35.0*	34.1*	34.2*
		SEM	0.3	0.1	0.2	0.3
1000	No Swim	mean	36.3	35.1*	34.8*	33.8*
		SEM	0.3	0.4	0.5	0.4
—	CWS	mean	36.4	30.6*	34.1*	35.1*
		SEM	0.1	0.7	0.6	0.5
500	CWS	mean	36.5	26.8*†	29.8*†	31.4*†
		SEM	0.2	0.6	0.7	0.6
1000	CWS	mean	36.7	25.4*†	27.7*†	28.9*†
		SEM	0.2	0.5	0.5	0.3

Significant difference (Dunnett Comparison, $p < 0.05$) from PRE (*) or CWS (†).

pairing of the 2°C CWS and the 500 μg/kg clonidine dose increased jump thresholds by 0.428 mA (107%), this potentiation appears to approximate additivity of the two analgesic effects.

EXPERIMENT 2

METHOD

The tail-flick latencies of male albino Sprague-Dawley rats were assessed [13] by applying a radiant heat source (IITC Analgesia Meter) 8 cm dorsal and 6 cm proximal to the tip of the tail. During a typical test session, three trials of tail-flick latencies were determined at 30 sec intervals. The intensity of the thermal stimulus was set so as to elicit stable baseline tail-flick latencies between 2 and 3 sec. To avoid tissue damage, a trial was automatically terminated if a response did not occur within 10 sec.

To determine the effects of clonidine upon CWS-induced increases in tail-flick latencies, 18 rats, matched on the basis of baseline tail-flick latencies over three days, were divided into three groups of six rats. The first group received clonidine (500 μg/ml normal saline/kg body weight, IP) and latencies were determined 60, 90 and 120 min thereafter. The second group underwent a 3.5 min swim in a 2°C temperature bath and tail-flick latencies were determined 30, 60 and 90 min thereafter. The third group received the 500 μg/kg clonidine dose 30 min prior to the swim; tail-flick latencies were determined 30, 60 and 90 min following the swim.

RESULTS

Table 3 summarizes the significant alterations in tail-flick latencies as a function of the baseline and post-treatment tests, $F(3,45)=25.10$, $p<0.001$, and for the interaction between groups and tests, $F(6,45)=4.20$, $p<0.002$. The effect among groups approached, but did not achieve statistical significance, $F(2,15)=3.07$, $p<0.077$. While CWS significantly increased tail-flick latencies over the post-treatment time course whether or not it was paired with clonidine, the clonidine injection at this dose failed to significantly alter latencies. The magnitude of CWS analgesia was significantly potentiated 30 and 60 min after the swim when paired with the 500 μg/kg clonidine dose.

EXPERIMENT 3

METHOD

The core body temperatures of 36 male rats were measured by inserting the rectal probe of a digital thermometer (Bailey Instruments) until a stable temperature was determined. Animals were assigned to one of the following groups and received: (a) 1000 μg/kg clonidine (1000 μg/kg normal saline/kg body weight, IP) 30 min before handling (no swim): n=6; (b) 500 μg/kg clonidine 30 min before handling: n=6; (c) a 3.5 min swim in a 2°C temperature bath: n=12; (d) 1000 μg/kg clonidine 30 min before the 2°C CWS: n=6; and (e) 500 μg/kg clonidine 30 min before the 2°C CWS: n=6. Core body temperatures were measured 30, 60 and 90 min after the CWS or no swim condition.

RESULTS

Table 4 summarizes the significant alterations in core body temperatures among groups, $F(4,31)=37.43$, $p<0.001$, across the pre and post-treatment groups and tests, $F(3,93)=179.75$, $p<0.001$, and for the interaction between groups and tests, $F(12,93)=24.42$, $p<0.001$. Significant decreases in core body temperature were observed across the post-treatment time course following both clonidine doses alone, CWS alone and CWS paired with each clonidine dose. The degree of CWS hypothermia was significantly potentiated when paired with either the 500 or 1000 μg/kg clonidine doses.

GENERAL DISCUSSION

Clonidine, an alpha-noradrenergic receptor agonist, dose-dependently increases jump thresholds, supporting previous reports of analgesia following systemic clonidine on the vocalization, tail-flick, writhing and yeast-paw tests [11, 15, 19]. The interaction between clonidine and CWS on the

jump test appeared to be additive when the 15°C CWS was paired with the 500 and 1000 μg/kg clonidine doses and when the 2°C CWS was paired with the 500 μg/kg clonidine dose. The potentiation noted following the pairing of the 1000 μg/kg clonidine dose with the 2°C CWS appeared to be larger than mere additivity. While tail-flick latencies failed to be significantly affected by a 500 μg/kg clonidine dose, this dose when paired with a 2°C CWS significantly potentiated the latter's analgesic effect on the tail-flick test.

These data provide further support for the involvement of the alpha noradrenergic receptor in the modulation of basal pain perception as well as analgesic processes. Phentolamine, an alpha-noradrenergic receptor antagonist, produces analgesia when applied directly into the nucleus raphe magnus [16, 17, 24] and hyperalgesia when applied intrathecally [20]. Intrathecal phentolamine also blocks the analgesic responses induced by intrathecal norepinephrine [22,23], morphine injected into the periaqueductal gray [32] or phentolamine injected into the nucleus raphe magnus [24]. Phentolamine however blocks both postsynaptic (Type 1) and presynaptic (Type 2) alpha-noradrenergic receptors, and manipulation of these receptor sub-types produce differential effects upon nociceptive processes. Stimulation of postsynaptic alpha-noradrenergic receptors with phenylephrine elicits hyperalgesia when applied directly into the nucleus raphe magnus [25] and elicits analgesia when applied intrathecally [22]. Like phentolamine, prazosin, a postsynaptic alpha-noradrenergic antagonist, elicits analgesia when applied directly into the nucleus raphe magnus [25]. In contrast, stimulation of presynaptic alpha noradrenergic receptors with clonidine elicits analgesia when administered either sytemically [11, 15, 19], directly into the nucleus raphe magnus [25] or intrathecally [22]. Yohimbine, a presynaptic alpha-noradrenergic antagonist blocks norepinephrine analgesia following intrathecal administration [22] and produces hyperalgesia when applied directly into the nucleus raphe magnus [25]. The analgesic stress response to CWS also appears to correlate with changes in central nervous system norepinephrine levels: acute exposure to CWS reduces brain norepinephrine [28, 29, 31] and induces analgesia [5]; chronic exposure to CWS fails to alter either brain norepinephrine [31] or pain thresholds [6]. Like CWS, foot shock reduces brain norepinephrine [30] and elicits analgesia that is sensitive to manipulations of the presynaptic alpha-noradrenergic receptor sub-type [11,12].

One can conclude from the present data that stimulation of the presynaptic alpha-noradrenergic receptor potentiates CWS analgesia by providing either excitation or disinhibition of the pain-inhibitory system mediating CWS. Alternatively, such presynaptic alpha-noradrenergic receptor stimulation may be acting to enhance the stressful consequences of CWS. To test the relative contributions of a given manipulation to alter either pain-inhibition per se or the stressors' consequences, a second physiological response to CWS, hypothermia, can be examined. If the manipulation that alters CWS analgesia is acting on the pain-inhibitory system mediating the response, one would expect changes in the analgesic, but not the hypothermic response to CWS. In this regard, CWS analgesia, but not CWS hypothermia, is reduced by repeated pre-exposure to the swims [6], by hypophysectomy [3], by D-phenylalanine [7], and, under certain parameters, by naloxone [9]. If the manipulation that alters CWS analgesia is acting on the stressful consequences of CWS itself, one would expect parallel changes in the analgesic and hypothermic responses to CWS. In this regard, both CWS analgesia and CWS hypothermia are reduced in rats neonatally treated with monosodium glutamate [2]. The potentiating effects of clonidine on both the analgesic and hypothermic responses following CWS suggest that systemic clonidine may be acting to enhance the stressful consequences of the CWS stressor. Whether other analgesic effects of clonidine following central and intrathecal administration can be attributed to its hypothermic effects is subject to further study as well as possible dissociations of CWS analgesia and CWS hypothermia following central or intrathecal administration.

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