Review article

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ENDOGENOUS SYSTEMS INVOLVED IN EXERCISE-INDUCED ANALGESIA

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Exercise-induced analgesia is a phenomenon discussed worldwide. This effect began to be investigated in the early 1970s in healthy individuals and rodents during and after an acute or chronic session of running or swimming. Thereafter, studies found this effect was also induced by resistance exercises. Over the years, many studies have demonstrated the importance of exercise-induced analgesia in relieving pain caused by different conditions, such as fibromyalgia, low back pain, neuropathy, and osteoarthritis. This review aims to provide the reader with an in-depth description of the main endogenous systems, substances, neurotransmitters, receptors and enzymes that are thought to be involved in the analgesic effect induced by exercise. Many hypotheses have been proposed to elucidate the mechanisms responsible for exercise-induced analgesia. One of the most accepted hypotheses has been the activation of several endogenous systems described as analgesics. Studies have demonstrated that during and after exercise different endogenous systems are activated, which release substances or neurotransmitters, such as opioids, nitric oxide, serotonin, catecholamines and endocannabinoids, that may modulate the pain perception.

K e y   w o r d s: *exercise, analgesia, pain, catecholamines, opioid system, nitric oxide, 5-HT, noradrenergic system, endocannabinoids, anti-inflammatory cytokines*

INTRODUCTION

For more than 30 years, studies have shown the analgesic effect produced by exercise in healthy subjects and chronic pain conditions and have expanded these studies to investigate the complex molecular mechanisms involved in exercise-induced analgesia (1, 2).

Although studies have found that exercise produces an analgesic effect, prescription remains difficult in some cases, since the optimal exercise parameters such as type and intensity are still not well defined (3, 4). Furthermore, not all types of pain conditions respond equally well to exercise-induced analgesia.

Exercise-induced analgesia has been reported during and after different types of exercise, such as aerobic, resistance, and isometric (1, 5). This effect is most prominent with higher intensity and longer duration of aerobic and isometric exercises. Few studies have investigated the different load of resistance-exercise on the nociceptive threshold. The first studies that investigated exercise-induced analgesia used mechanical and electrical stimuli to evaluate this effect. Later studies used thermal stimuli, but the results were somewhat inconsistent (3, 6, 7). Regarding the influence of age on this effect, studies using different modes of exercise have found pain and functional improvement in older adults with chronic pain (4, 8). In animals, a study investigated the effects of aging on walking-induced analgesia using a formalin-induced paw licking test. The authors found that the analgesic effect induced by walking was age-dependent with the oldest mice exhibited the least

analgesia (4 > 24 > 48 weeks) (9). Studies investigating sex differences in exercise-induced analgesia have produced inconsistent results. A study showed that only women reported increase in nociceptive thresholds after two brief maximal handgrip contractions. However, when pain ratings were evaluated, both men and women reported decreased pain (10). In addition, other studies have found that isometric contractions increased the nociceptive threshold in men and women (11, 12). Comparing the sex differences in the nociceptive threshold of older individuals, a study found that older women reported greater pain sensitivity, higher pain ratings, and larger reductions in pain ratings than men after isometric exercise (5). Independent of athletic status, a study showed that treadmill running for 10 min at 85% induced reductions in ratings of pain intensity and unpleasantness during a cold pressor test in women but not in men (13).

Although exercise-induced analgesia is well documented in the literature, there are several hypotheses that attempt to unravel the cause of this effect. The most accepted of these hypotheses is the activation of endogenous systems, such as the opioidergic, nitrergic, serotoninergic, noradrenergic, and endocannabinoidergic systems and of anti-inflammatory cytokines. Additional hypotheses have been proposed, such as activation of high threshold motor units, activation of the primary motor cortex and corticospinal tracts, and interaction of the cardiovascular and pain modulating systems. In this sense, the present study aimed to determine which of the previously implicated endogenous systems are involved in exercise-induced analgesia.

ENDOGENOUS SYSTEMS INVOLVED
IN EXERCISE-INDUCED ANALGESIA

Opioidergic system

The discovery of endogenous opioids started after Hughes and Kosterlitz (14), in 1974, isolated enkephalins from pig brain. Around the same time, Simantov and Snyder (15) found a similar substance in calf brain and named it 'endorphin', which is an abbreviation of 'endogenous morphine'.

The endogenous opioid system includes a large number of opioid peptides, such as endorphins, enkephalins, and dynorphins, which are derived from the precursor polypeptides, pro-opiomelanocortin, proenkephalin, and prodynorphin, respectively (16). These peptides bind to opioid receptors; three primary opioid receptor types mediate analgesia and are designated μ, κ, and δ. The interaction between G protein-coupled opioid receptors and their effectors can be direct or involve intermediate or other indirect effector pathways (17). The activation of inward-rectifying K^+ channels, the inhibition of voltage-dependent Ca^{2+} channels and the inhibition of adenylyl cyclase are direct effects of the activation of G protein-coupled opioid receptors (17). Conversely, these opioid receptors can also activate phospholipase C (PLC), the mitogen-activated protein kinase (MAPK) cascade, and large conductance Ca^{2+}-activated K^+ channels by utilizing intermediary messenger systems (18). Modification of the Ca^{2+} and K^+ channel currents by opioid receptors can lead to a decrease in neuronal excitability, a decrease in the neuronal firing rate, and inhibition of neurotransmitter release (17). These receptors are largely located in all areas of the central nervous system (CNS), including areas involved in analgesia (the brainstem, medial thalamus, spinal cord, hypothalamus, and limbic system). Furthermore, opioid receptors have also been identified in the peripheral nervous system (15).

Basbaum and Fields (19) proposed that the analgesic action of endogenous results from activation of excitatory connections between the periaqueductal gray area (PAG) and the nucleus raphe magnus. Nucleus raphe magnus neurons, in turn, project, *via* a pathway in the dorsal part of the lateral funiculus (DLF) of the spinal cord to the region of nociceptors in the spinal dorsal horn, and its trigeminal equivalent, the nucleus caudalis. These raphe-spinal neurons selectively inhibit dorsal horn nociceptive neurons, including interneurons and a population of rostrally projecting spinothalamic and spinoreticular neurons (20).

In addition, several studies have demonstrated an increase in the plasma levels of endogenous opioids, mostly β-endorphin, during and after exercise. They found an increase in β-endorphin levels during aerobic exercise with an intensity of 85% of the maximum heart rate in women trained to exercise to exhaustion (21, 22). Furthermore, these studies have demonstrated that this increase is directly related to the intensity applied. High β-endorphin levels were found after 20 min of stationary bike exercise, only increasing to 80% of VO_{2Max} when compared to 40% and 60% VO_{2Max}. In addition, other studies found that β-endorphin levels change after running between $40 – 70\%$ VO_{2Max} (21, 22). However, another study that compared a progressive exercise protocol of 60%, 70% and 80% VO_{2Max} for 1 hour demonstrated that each intensity similarly produced a significant increase in β-endorphin levels (23). Similar to aerobic exercise, studies also have found an increase in β-endorphin levels after resistance exercise. The first study compared burst activity resistance exercise (weight lifting) to aerobic exercise (treadmill) in five males and found a similar increase in β-endorphin levels between the exercises (24). In addition, an increase in plasma β-endorphin concentrations was found after heavy-resistance exercise in eight healthy males (25). However, similar to studies

using aerobic exercise, there were contradictory results from studies performed using resistance exercise. There was no difference between the β-endorphin levels of six resistance-trained athletes who had participated in a three-set series of eight repetitions of an isotonic exercise performed at 80% maximal effort (26). Furthermore, these same authors found in another study that β-endorphin levels were reduced after three sets of eight repetitions of isotonic resistance exercise at 80% of one repetition maximum in males and females when compared to the levels before exercise (27). However, none of these previously described studies associated these increases with analgesia evaluation.

Thus, after studies demonstrated the involvement of endogenous opioids in analgesia and that exercise produces an increase in β-endorphin levels, several studies have begun to investigate the participation of endogenous opioids in exercise-induced analgesia.

In a study that evaluated 8 men and 3 women who were subjected to exercise protocols (20 min of leg and arm exercises) and transcutaneous nerve stimulation (TENS, low frequency) exercise was shown to significantly increase the dental pain threshold of the subjects, which gradually returned to baseline levels 50 min after exercise. The analgesic effect found after 20 min of arm and leg exercise was reversed by naloxone (0.8 mg, a non-selective opioid receptor antagonist) injected (i.v.) (28).

A double-blind study, performed with eight patients diagnosed with symptomatic myocardial ischemia and nine with asymptomatic myocardial ischemia was compared during physical exercise under naloxone (6 mg, i.v.) or placebo. Ischemic (a tourniquet on the forearm, under control of transcutaneous PO_2) and electric nociceptive thresholds (intracutaneous electrode placed in the finger with the electrical stimulus under computer control and two-interval forced-choice psychophysical technique) and plasma β-endorphin levels were assessed before, during and following exercise. Results indicated that ischemic and electrical pain thresholds were higher in the asymptomatic patients compared to symptomatic patients at baseline. A moderate, but statistically insignificant, increase in pain threshold was found following exercise. Naloxone i.v. administration attenuated ischemic pain threshold following exercise but had no effect on electrical pain threshold. Plasma β-endorphin levels were found to increase during exercise, with significantly higher increases in the asymptomatic patients compared to symptomatic patients. In addition, naloxone administration also attenuated the β-endorphin increase observed in the asymptomatic patients (29).

A study that investigated the influence of exercise on the nociceptive threshold using multiple pain stimuli showed that cold pressor pain did not influence 12 runners after 10 km run at 85% of maximal aerobic capacity; however, the thermal and ischemic pain were attenuated in the same participants following exercise and this effect was associated with increase of β-endorphin plasma levels. In addition, it was found that i.v. injection of naloxone (8 mg) reversed the post-run analgesic response for ischemic stimulation but not for thermal stimulation (30). Furthermore, sensory-decision theory analysis was employed to assess both the discriminability of pain stimuli and pain report criterion of three different stimuli (thermal, ischemic and cold pressor). The discriminability represents how well a person can distinguish between different intensities of painful stimuli, while the pain report criterion refers to the person's willingness to report a stimulus as painful. The results revealed that thermal discriminability and ischemic discriminability were significantly reduced following running, indicating that an analgesic response had occurred (30).

In addition, besides pain stimuli, the antagonist dose also influenced the analgesic response found after exercise. A study demonstrated that treatment with 2 mg naloxone completely

blocked the analgesic effect, caused by a 1-mile run, on pressure pain thresholds in 9 men and 6 women when compared to a 10 mg dose (31). However, another study demonstrated that high-dose naloxone (20 mg) did not influence the analgesic response, evaluated by the response to electrical stimuli applied to the dental pulp or pressure tests applied to the finger, in ten healthy active men that underwent exhaustive exercise on an ergometric cycle (32).

The most compelling evidence to support the theory of exercise-induced analgesia and the involvement of the endogenous opioid system have been provided by animal experimentation. Most of these studies investigated whether exercise-induced analgesia is mediated by endogenous opioid mechanisms using swimming and running as primary exercise stimuli.

The pioneering study investigating the systemic involvement of endogenous opioid systems in exercise-induced analgesia showed that different doses of naloxone (0, 1, 5, 10 and 20 mg/kg, subcutaneously (s.c.)) administered at weekly intervals produced a dose-dependent reduction of up to a maximal reduction of 50% at 20 mg/kg in the swim-induced analgesia in rats, measured by flinch-jump thresholds (33). In addition, another study evaluated the influence of various cold-water (2°C) swim parameters on exercise-induced analgesia. Male rats were exposed to (i) various durations of cold-water swims, (ii) intermittent versus continuous cold-water swims, and (iii) 60 consecutive cold-water swims in naltrexone (an opioid receptor antagonist) and saline conditions. Naltrexone subcutaneously administered 10 min before swimming partially antagonized continuous cold-water swim-induced analgesia, but at only high doses of naltrexone (21 mg/kg). However, at lower doses (14 mg/kg), naltrexone significantly antagonized intermittent cold-water swims and enhanced the analgesic response produced by 60 consecutive swims. These results demonstrated that naltrexone differentially influences cold-water swim-induced analgesia depending upon specific parameters of the exercise condition, including the duration of the swim, whether the swim was intermittent or continuous, or whether a large number of consecutive cold-water swims were completed (34). However, another study demonstrated that the swim-induced analgesia in female mice, evaluated by the response to a hot plate test, was blocked by low-dose (100 µg/kg, s.c.) naloxone administered 1 hour prior to swimming. Furthermore, these authors found that the analgesic effect produced by exercise was greater than the analgesia obtained upon injection of 15 mg/kg of morphine (35). In addition to the dose, the pre-administration time and the type of antagonist were also shown to be important factors in cold-water swim-induced analgesia. Naloxone had a more rapid effect, onset and shorter half-life than naltrexone.

The duration of exercise can also influence the analgesic response. A study compared a female mouse group that swam for 15 s with another group that swam 3 min. Analgesia was induced in both groups; however, when parallel groups were pretreated with naloxone (5 mg/kg, s.c.), analgesia was blocked in only the 3-min-swim group (36). These studies suggested that the swim duration influences the nature of analgesia and that there appear to be multiple analgesic systems (opioid and non-opioid) involved in this type of analgesia.

Studies investigating endogenous mechanisms involved resistance exercise-induced analgesia in animals is limited. Only one study using a weightlifting exercise model demonstrated the participation of the endogenous opioid system in the analgesic response. In this study, the animals underwent an acute resistance exercise protocol (15 sets of 15 repetitions) and training protocol (3 sets of 10 repetitions, three times per week for 12 weeks); in both groups, the exercise intensity began at 75% maximal repetition. The nociceptive threshold was measured by a paw-withdrawal test, which demonstrated that analgesia occurred only after acute resistance exercise, and this effect was reversed by naloxone (2 mg/kg) preadministration (37).

Regarding isometric exercise, although some studies have revealed that it induces reduction in pain ratings and increase in pain threshold (10, 39, 40), only one study investigated opioid endogenous levels after isometric exercise. This study did not find alterations in beta-endorphin levels in six elite kayak paddlers and 14 untrained subjects after an isometric knee-extension exercise (41). Furthermore, these results were not associated with nociceptive threshold evaluation.

An important study found a longer tail-flick latency in female mice that swam for 3 min in 32°C water compared to non-swimming controls, and this result correlated with a reduction of the [3H] leu-enkephalin (LE) binding in the brain homogenates of exercise-induced mice. The authors suggest that the reduced LE binding may be due to the occupation of a proportion of the opioid receptor population by an endogenous ligand, which is consistent with the interpretation of the involvement of endogenous opioids in the observed increased in tail-flick latency (42). However, no antagonist was used and no additional experiments were performed to confirm this hypothesis.

Exercise-induced analgesia was also found to be the same in both disease and pain animal models. Male spontaneously hypertensive and normotensive rats were trained to spontaneously run in running wheels. After 3 to 4 weeks of training, the pain sensitivity, measured by a squeak threshold to electrical stimulation, was reduced in both rats. Furthermore, naloxone intraperitoneal administration decreased squeak thresholds to baseline levels, indicating the involvement of the endogenous opioid system (43).

Low-intensity treadmill exercise for 5 consecutive days reduced chronic muscle pain induced by acidic saline solution in rats. This analgesic effect, evaluated by the response to von Frey filaments, was attenuated by naloxone intraperitoneal preadministration, demonstrating that the endogenous opioid system also participates in exercise-induced analgesia in the chronic muscle pain model (44). In addition, another study demonstrated that regular moderate aerobic exercise reversed neuropathy-induced thermal and tactile hypersensitivity and increased endogenous opioid (β-endorphin and met-enkephalin) content in the PAG and in the rostral ventromedial medulla (RVM); these regions are important in descending pain modulation. Furthermore, the analgesic effect induced by moderate aerobic exercise was reversed by naloxone and naltrexone, systemically or intracerebroventricularly administered, suggesting a peripheral and central involvement of opioid receptors (45).

In addition, another study demonstrated that the naloxone pretreatment (injected in the right tibiofemoral articular space) reversed the analgesia in Wistar rats with acute knee synovitis that were subjected to jumping-in-water exercise with a 50% overload (46). Opioid receptors located in peripheral nervous terminals may be activated by exogenous and endogenous opioids expressed in immune cells to produce significant analgesia (47).

The release of endogenous opioids by the adrenal gland may be another explanation for exercise-induced analgesia. A study demonstrated that analgesia was induced by a 5-day swimming period in male mice subjected to acetic acid-induced abdominal writhing but was not induced in mice with bilateral adrenalectomy or naloxone pretreatment (48).

As most studies have used naloxone or naltrexone, which are non-selective opioid receptor antagonists, it is difficult to locate a specific opioid receptor involved in exercise-induced analgesia. In addition, only one study investigated the involvement of opioid receptors in exercise-induced analgesia at

the central level (45). In most studies, especially with humans, naloxone was administered systematically and in some studies, it was discovered that beyond the reversal of analgesia, a reversal of euphoria also occurred, which suggests that its effect was systemic.

Despite being one of the first mechanisms described in exercise-induced analgesia, several studies were unable to elucidate the role of endogenous opioids in this effect, which suggested the involvement of other endogenous mechanisms.

Nitrergic System

Another important endogenous candidate for exercise-related analgesia is the nitric oxide (NO) system. Some studies have demonstrated its analgesic effect and an increase in the levels of nitrate in the plasma after exercise (49, 50). NO is a soluble gas continuously synthesized in mammalian cells as a by-product of the conversion of its physiological precursor, the amino acid L-arginine, to L-citrulline. This reaction is catalyzed by one of three isoforms of enzymes known as NO synthase (NOS). Two of the NOS enzymes, specifically endothelial NOS (eNOS) and neuronal NOS (nNOS), are calcium-dependent and constitutively produced at relatively low levels of NO. The inducible NOS isoform (iNOS) is expressed for a longer period of time upon activation by a variety of factors, including exercise (51). Once synthesized, NO can diffuse within the same cell or neighboring cells, where it binds to the heme group of soluble guanylyl cyclase to generate $_c$GMP from GTP (52). A study showed that activated $_c$GMP promotes the opening of K_{ATP} channels, which may result in analgesia (53). Thus, by intracellular mechanisms, the analgesic effect of NO is produced by the NO/cGMP/K_{ATP} pathway.

Several factors are responsible for the increase of NO production during resistance exercise. In rodent skeletal muscle, nNOS, eNOS and inductive NOS isoforms are highly expressed in muscle fibers and are activated by exercise. The increase of shear stress by increased blood flow and muscle contraction induced distortion of resistance vessels, stimulates eNOS and nNOS (54). In addition, microdamage to myofibrils during muscle contractions releases/stimulates inflammatory cells, which activate iNOS. Red blood cells release ATP in low oxygen environments and in response to deformation due to muscle contractions. ATP binds to purinergic receptors on the endothelium, leading to eNOS activation and NO production (55). Furthermore, during exercise, there is an increase in NO levels, which has been implicated in metabolic control *via* effects on blood delivery, glucose uptake, oxidative phosphorylation, contractility, and excitation-contraction coupling of the skeletal muscle (56).

Additionally, an increase in blood NO levels was correlated with the reduction of migraine pain severity, frequency and duration in women that underwent a regular long-term aerobic exercise protocol that consisted of exercise periods of 1 hour/day, 3 days/week for eight weeks (57). Furthermore, NO has been demonstrated to have the ability to reduce the nociceptive activity of the peripheral and central nervous system after exercise. Studies found the involvement of NO/cGMP/K_{ATP} in the analgesia produced by acute aerobic resistance exercise in rats (58, 59). In these studies, the acute aerobic exercise protocol involved the animals running on a treadmill for an average time of 59 min at a progressive velocity until the onset of fatigue, and the resistance exercise protocol involved a weight-lifting exercise model that consisted of the animals performing 15 sets of 15 repetitions with a load at 65% to 75% of 1 repetition maximum. Immediately after exercises, the mechanical and thermal nociceptive threshold was increased, which was measured by paw-withdrawal and tail-flick tests. This effect was reversed by

specific inhibitors of NOS isoforms (aminoguanidine an iNOS inhibitor; $_L$-NIO, an eNOS inhibitor and $_L$-NPA, a nNOS inhibitor) as well as peripheral (s.c.) and central: (i.t. and i.c.v.) preadministration of cGMP and K_{ATP} (ODQ and glybenclamide). Furthermore, plasma and cerebrospinal fluid (CSF) nitrite levels were increased after exercise, and the expression of NO were increased in the dorsolateral and ventrolateral PAG. These authors suggest that dorsolateral and ventrolateral PAG contain a column of NOS-containing cells, which during exercise may release NO that can participate in the inhibitory modulation of pain (60). However, another study found that the pretreatment of unspecific inhibitors of NOS (L-NAME and L-NA, intraperitoneally (i.p.)) potentiated the swim-induced analgesia measured by tail-flick and jump tests in rats (61). Studies demonstrated that nitric oxide synthase inhibitors, such as L-NAME, may act as partial agonists stimulating instead of inhibiting NOS and guanylyl cyclase, which may contribute to the observed dual effect of NO in the nociceptive system (62, 63).

NO may have a dual role in the regulation of pain processes, i.e., it can mediate nociception or induce an antinociceptive effect. This dual effect occurs in both the central and peripheral nervous systems (64). Many studies have focused on understanding the dual effect (pro or antinociceptive) of NO, and these data indicate that this effect is dependent of NO levels and the phase of the nociceptive process (64). L-arginine, a biological precursor of NO, at a 0.1 – 1 ug dose i.pl. per paw, enhanced second-phase formalin-induced behavioral nociception, whereas at 3 ug per paw, it did not have significant effects and conversely, at 10 ug per paw, it produced antinociception resulting in a bell-shaped dose-response curve. In addition, L-NAME (a L-arginine inhibitor) i.pl., at 1 ug per paw, produced antinociception in this phase and considerably reduced the increase in second-phase nociception elicited by low doses of L-arginine (65).

Additionally, another study showed that a low dose of intrathecal administration of NO donors produced antinociception, while higher doses enhanced (i.e., caused a pronociceptive effect) or had no effect on the mechanical allodynia evoked by chronic ligature of the sciatic nerve in rats. The authors suggested that NO produces a depression in the calcium current in the same areas of nociceptive impulse conduction. However, the increase in NO concentration may in turn increase the amplitude of calcium currents, facilitating nociception (66). Rocha *et al.* (67) suggested that endogenous NO is an important mediator involved in the development of zymosan-induced arthritis, whereas the pharmacological administration of NO can inhibit the ongoing nociceptive phenomena. These authors demonstrated that the inhibitors of NOS decrease articular inflammatory pain induced by zimosan; however, this inhibitory effect was observed only when the inhibitors were administered before the induction of arthritis. Furthermore, it was observed that administration of NO donors induced an analgesic effect, and this effect was observed for the drugs administered after the injection of zymosan. Although these studies have revealed the dual role of nitric oxide on the nociceptive system, the studies on exercise previously cited have found that only this substance induces analgesia.

Serotonergic system

In the 1930s, Erspamer began to study the distribution of enterochromaffin cells, which stained with a reagent for indoles. The highest concentrations were found in the gastrointestinal mucosa, followed by the platelets and the CNS (68). Hence the unknown indole was named entramine. After that, Page and his colleagues at Cleveland clinic isolated and characterized a vasoconstrictor substance released from clotting blood, which

was called serotonin (68). Rapport deducted that the active moiety was 5-hydroxy tryptamine, which was isolated as serotonin by Page. In 1952, Erspamer and Asero identified entramine as 5-HT. 5-HT is autacoids as well as important neurotransmitter in CNS and peripheral nervous system (PNS) (68). The serotonergic system is known to modulate pain, mood, emotion, sleep and so it is implicated in the control of numerous behavioral and physiological functions. 5-HT exerts its multiplicity of physiological effects through an unsurpassed diversity of receptors (69).

The involvement of 5-HT in the descending control of pain has long been recognized (70). The pharmacological evidence showed that electrical stimulation of the nucleus raphe magnus (NRM) increases the release of metabolites of 5-HT in the medullary dorsal horn cells in the rat spinal cord (71). Studies demonstrated that electrical or chemical stimulation of the NRM and the surrounding nucleus reticularis paragigantocellularis produced analgesia, which was antagonized by intrathecally administered 5-HT receptor blockers and was accompanied by the efflux of 5-HT from the spinal cord (72). 5-HT is generally thought to be an inhibitory neurotransmitter in the dorsal horn cell. It is involved not only in modulating pain transmission signals in the dorsal horn but is also released from this site. However, a study found that $5-HT_3$ receptors are involved in hyperalgesia and tolerance induced by morphine. In that study, the authors demonstrated that systemic or intrathecal injection of a $5-HT_3$ antagonist significantly prevented or reversed these symptoms (73). In addition, this group had previously demonstrated that morphine is strongly regulated by the expression of the *Htr3a* gene in various central nervous system regions including the amygdala, dorsal raphe, and periaqueductal gray matter, which have been linked to opioid dependence (74).

5-HT has been demonstrated to be involved in exercise-induced analgesia by several studies evaluating humans and animals. Studies found that analgesia induced by intermittent cold-water swims (18 pairs of 10-s swims and 10-s recovery periods at 2°C) in rats, was reduced by both systemic and intrathecal $5-HT_2$ antagonist receptor (methysergide, 5 – 10 mg) administration (75). Additionally, another study also demonstrated the involvement of serotonin in the analgesia induced by high-intensity extended swimming exercise in mice, which was reversed by pretreatment with ρ-chlorophenylalanine methyl ester (PCPA, an inhibitor of serotonin synthesis, 100 mg/kg, i.p.) (48). These results suggested that $5-HT_2$ participates in exercise-induced analgesia at the peripheral and spinal levels.

Exercise produces an increase in (5-HT) concentrations in most brain areas, including areas involved in pain control (76). In addition, a study demonstrated that treadmill running exercise results in the release of 5-HT in layers II, III, IV, V of the dorsal horn (77). Furthermore, rats with sciatic nerve transection that trained on a treadmill presented a higher serotoninergic immunoreactivity in the neuronal cytoplasm of NRM, the area also involved in the control of pain, which was associated with an increase in the mechanical nociceptive threshold (78). Although, these areas (dorsal horn and NRM) are importantly involved in the descending control of pain, the nociceptive threshold was not evaluated.

Exercises have been the primary mode of treatment for low back disorders, aiming to achieve pain reduction. Studies showed that spinal stabilization and treadmill walking exercises produced a significant increase in plasma 5-HT levels in participants with chronic low back pain (79, 80). However, the increase in 5-HT levels after exercise was not associated with the pain response of the participants in any of these studies..

However, in women with fibromyalgia, a study found that aerobic walking exercise three times a week for 20 weeks significantly increased 5-HT levels and its main metabolite 5-hydroxindolacetic acid (5HIAA) when compared with baseline levels; there were no differences in the pain visual analog scale values (81).

Noradrenergic system

The noradrenergic system is an essential system that becomes activated during exercise to exert important functions, such as cardiovascular control, fuel mobilization and release of hormones and neurotransmitters (i.e., catecholamines) (82). Furthermore, the noradrenergic system may also participate in exercise-induced analgesia. Catecholamines may modulate the nociceptive pathway by activation of α2-adrenergic receptors (α2-ARs) (83). The α2-ARs receptors are divided into 3 subtypes α2A, α2B, and α2C. The α2A and α2C-ARs are Gi/Go protein coupled, which inhibits cyclic adenosine monophosphate production; the resulting opening of K^+ channels and closing of voltage-gated Ca^{2+} channels, in turn, results in hyperpolarization and a reduced rate of firing of excitable cells (84). α2-ARs have also been found in different areas involved in pain control, for example, the periaqueductal gray, *Locus coeruleus* and dorsal root ganglion (DRG) (85).

Few studies have investigated the involvement of the noradrenergic system in exercise-induced analgesia, all of which were performed using rodents. The first study that evaluated the participation of this system was conducted in 1985, and it examined whether acute or chronic desipramine (a selective inhibitor of the norepinephrine transporter) intraperitoneal injection could potentiate cold-water swim-induced analgesia in mice. However, the authors found that neither acute nor chronic desipramine treatment altered the analgesic effect induced by swimming (86).

Other studies using different antagonist pretreatments also demonstrated the involvement of the noradrenergic system. A study found that idazoxan, prazosin and yohimbine (α1 and α2-adrenergic antagonists) systemic and i.c.v. preadministration attenuated warm-water swim-induced analgesia in mice. Furthermore, the warm-water swim-induced analgesia was potentiated by clonidine (α2-adrenergic receptor agonist) and noradrenaline intracerebroventricular preadministration (87). Thus, this work suggests involvement of the peripheral and central levels of the noradrenergic system, *via* α1 and α2-adrenergic receptors in this effect. In addition, other studies have found that clonidine analgesia was potentiated by swimming, reinforcing the participation of α2-adrenergic receptors (88, 89).

The noradrenergic system was also found to be involved in the analgesia induced after running on a treadmill and resistance exercise in rats. A study demonstrated that this analgesic effect was reversed by α2, α2A, and α2C-adrenergic receptors antagonist (yohimbine, rauwolscine and BRL 44408) subcutaneous preadministration and by guanethidine (a selective inhibitor of transmission in adrenergic nerves) pretreatment. Furthermore, the analgesic effect produced by running was not observed in α2-AR knockout mice. In addition, when yohimbine was administered intrathecally or intracerebroventricularly, it did not alter antinociception induced by aerobic and resistance exercise protocols; α2-ARs expression in the rat brain did not change after both exercise protocols, revealing peripheral involvement of the noradrenergic system (90).

Endocannabinoid system

The endocannabinoid system is comprised of two G protein-coupled membrane receptors (cannabinoid CB_1 and CB_2 receptors) that are negatively coupled to adenylate cyclase and positively coupled to mitogen-activated protein kinase (91). These

receptors have been found in the CNS, including structures that participate in the descending control of pain, such as the PAG, RVM and the dorsal horn of the spinal cord, peripheral nervous system in DRG and immune cells (91, 92). Both receptors, when activated by their endogenous ligands (endocannabinoids), such as anandamide (AEA) and 2 arachidonoylglycerol (2-AG), which are often accompanied in tissues by non-cannabinoid receptor congeners, such as palmitoylethanolamide (PEA) and oleoylethanolamide (OEA), promote hyperpolarization and a reduction in the rate of firing of excitable cells, suppressing neurotransmitter release, and a reduction in the nociceptive impulse (93). The endocannabinoid AEA is hydrolyzed by the enzyme fatty-acid amide hydrolase (FAAH), whereas the other endogenous cannabinoid receptor ligand, 2-AG, is degraded by the enzyme monoacylglycerol lipase (MGL) (93). FAAH also partly regulates the levels of PEA and OEA.

Studies have demonstrated the participation of the endocannabinoid system in important responses during exercise such as muscle vasodilatation; euphoria and bronchodilation (94). The endocannabinoid system may also be involved in movement control since cannabinoid CB_1 and CB_2 receptors were found in the basal ganglia and cerebellum (91, 95). Cannabinoid receptors also have been found in the hypothalamus and may participate in the control of thermoregulation during exercise (96). Furthermore, another study suggested the involvement of this system in the aerobic capacity, after demonstrating that pretreatment with Rimonabant (a cannabinoid CB_1 receptor antagonist) reduced the running speed and distance in female high runner lines (97). In addition, the same studies found an increase in endocannabinoids after exercise. In 2013, a study in men provided the first evidence that the endocannabinoid system was activated by exercise, as evidenced by significant elevations in circulating plasma AEA levels but not in 2-AG levels following acute cycling or running (94). Then, another study investigated the influence of intense exercise (60 min at 55% followed by 30 min at 75% W_{max}), with 11 trained male cyclists, on endocannabinoid plasma levels. The authors found significant elevations in AEA levels as well as in PEA and OEA levels during exercise and 15 min recovery, whereas 2-AG concentrations remained stable (98).

In addition, endocannabinoid responses to different intensities of exercise were examined, and significant elevations in AEA levels following moderate-intensity treadmill exercise were found but not at lower or higher intensities of exercise (99). In an animal study, Hill *et al.* (100) found that eight days of free access to running wheels increased the tissue content of AEA in the hippocampus. All of these previous studies indicated significant elevations in endocannabinoids levels following exercise.

However, only a limited number of studies have been conducted examining the involvement of endocannabinoid system in exercise-induced analgesia.

A study performed with fifty-eight participants who participated in submaximal isometric exercise demonstrated that a reduction in the pressure-pain threshold after exercise was associated with an increase in AEA, 2-AG, PEA and OEA plasma levels (101). In another study by the same group, which used a similar methodology, the authors found that naltrexone prevented the increase of AEA and OEA plasma levels after isometric exercise, whereas the increase of 2-AG and 2-OG plasma levels was not prevented. The authors suggested that 2-AG and 2-OG could contribute to nonopioid exercise-induced analgesia and that the opioid system may be involved in the increase of AEA and OEA following exercise (102).

In addition, studies with rats have shown that the analgesic effect induced by running and resistance exercise was reversed by cannabinoid receptor antagonists and potentiated by endocannabinoid metabolizing enzyme and anandamide reuptake inhibitors, when they were pre-administered systemically (s.c.) and centrally (i.t. and i.c.v.) (103, 104). Furthermore, these studies found an increase in cannabinoid CB_1 receptor expression levels in the brain (PAG) and in the endocannabinoid plasma levels after both exercises. This indicates that acute running and resistance exercise affect the endocannabinoid system, which may participate in exercise-induced analgesia at the peripheral and central levels (103, 104). A study with mice also revealed the involvement of peripheral cannabinoid CB_1 and CB_2 receptors in the antinociception induced by acute long-distance running (105). In addition, another study with mice found that AMP-activated protein kinase is an intermediate effector in endocannabinoid-mediated exercise-induced by antinociception (106). This work showed that hyperalgesia induced by the formalin test was reduced after exercise only in wildtype mice, while exercise had no effect on nociception in $AMPK\alpha2$ knockout mice. Furthermore, the serum levels of AEA were increased after exercise in both wildtype and $AMPK\alpha2$ knockout mice, in association with decreased expression of FAAH and increased expression of the cannabinoid CB_1 receptor in the spinal cord. Additionally, treatment with the cannabinoid CB_1 receptor inverse agonist (AM251) prior to treadmill running reversed exercise-induced antinociception in wildtype mice. However, the combination of AM251 with AICAR (AMPK activator) restored the analgesic effect induced by exercise, indicating that AMPK affects exercise-induced antinociception downstream of endocannabinoids (106).

Anti-inflammatory cytokines

Anti-inflammatory cytokines are immunoregulatory molecules that control the proinflammatory and nociceptive responses. Major anti-inflammatory cytokines include the interleukin (IL)-1 receptor antagonist, IL-4, IL-6, IL-10, IL-11, and IL-13. These cytokines act in concert with specific cytokine inhibitors and soluble cytokine receptors to regulate the human immune response. Exercise reduced brain inflammation and this effect was associated with increase in IL-10 and reduction in proinflammatory cytokines IL-1β and tumor necrosis factor-α (TNF-α) (107).

In addition to endogenous analgesic substances, recent studies have gained attention by revealing the participation of anti-inflammatory cytokines in the mechanism of exercise-induced analgesia. A study found that regular physical activity altered the macrophage phenotype to increase IL-10 and prevent chronic muscle pain in mice. In addition, the authors demonstrated that the blockade of IL-10 systemically or locally prevented the analgesia in physically active mice, suggesting that regular physical activity increases the percentage of regulatory macrophages (M2, which secrete anti-inflammatory cytokines) in muscle tissue and that IL-10 is an essential mediator in the analgesia produced by regular physical activity (108). An earlier study had already found that exercise training inhibits inflammation in adipose tissue *via* both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in obese mice. However, proinflammatory cytokine release was not investigated (109).

Besides IL-10, IL-4 levels were increased in the spinal cord of mice with peripheral nerve injury exercised for 2 weeks. These levels were associated with increase in M2 and reduction in brain-derived neurotrophic factor, β-nerve growth factor, and glial cell activation (110), which are associated with sensitization of the nociceptive response.

Glia cells are involved in nociception genesis, especially in chronic conditions (111). This cell population is mainly composed of astrocytes and microglia (112), which when

activated are responsible for the release of pro-inflammatory cytokines that play a relevant role in the transmission of nociceptive impulses (113). Gong *et al.* (114) evaluated the role of microglia after infant peripheral nerve injury and the effect of exercise on the delayed-onset of neuropathic pain in rat pups. They found that exercise shifted spinal cord microglia polarization to the M2 phenotype and reduced neuropathic pain. In addition, IL-10 increased and TNF-α (an anti-inflammatory cytokine) decreased after exercise. The intrathecal injection of the IL-10 antibody reduced exercise-induced analgesia. Thus, the exercise was effective in the treatment of delayed adolescent neuropathic pain *via* the modulation of microglial polarization.

Others substances may also be associated to exercise-induced analgesia. Studies have demonstrated that exercise induces increase of brain-derived neurotrophic factor (BDNF)

(115,116). Although this protein is associated with central hypoxemia and fatigue induced by exercise (115, 116), evidences may support its involvement in the analgesic effect produced by exercise. A study demonstrated that midbrain infusion of BDNF decreased the behavioral paw flinch response to subcutaneous formalin injection and this effect was accompanied by an augmentation in serotonergic activity within the brain and spinal cord of rats (117). In addition, the analgesic effect produced by BDNF was reversed by naloxone preadministration (117). Furthermore, when injected within the PAG, dorsal raphe and spinal cord, BDNF increased the β-endorphin levels (118). Thus, these studies suggesting a modulatory effect of BDNF on the endogenous substances involved in the modulation of nociceptive information and supports the hypothesis of the involvement of this protein in the exercise-induced analgesia.
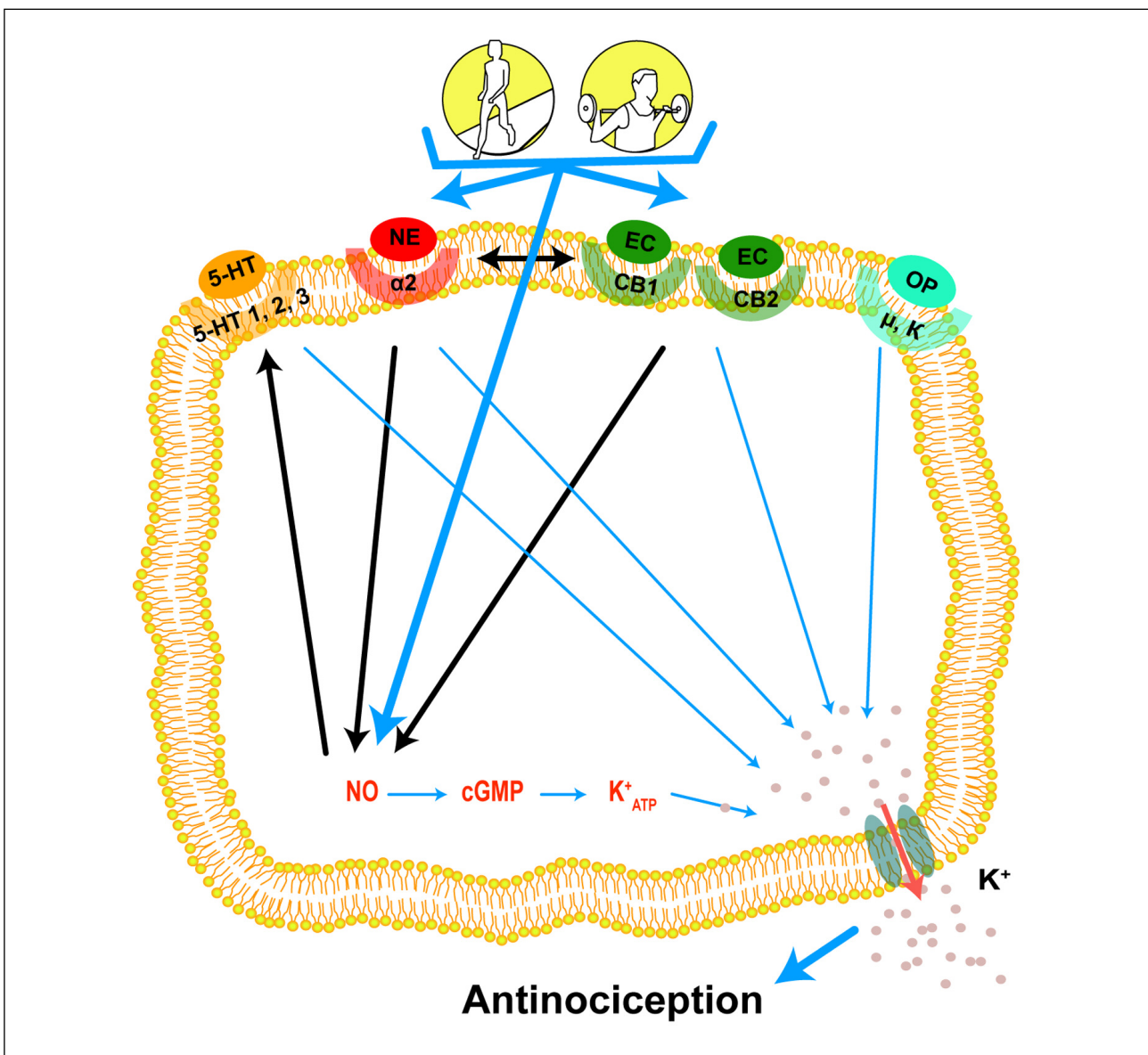

Fig. 1. Activation of endogenous systems during exercise-induced analgesia. During and after aerobic and resistance exercise occurs activation of NO/cGMP/K^+_{ATP} pathway, opioidergic, serotonergic, noradrenergic and endocannabinoids systems with consequent release of opioids (OP), serotonin (5-HT), norrpinephrine (NE) and endocannabinoids (EC), which will activate α2 adrenoreceptors, cannabinoid receptors type 1 and type 2 (CB$_1$ and CB$_2$), serotonin (5-HT1, 2, 3) and opioids (μ, K) receptors, resulting in the hyperpolarization of nociceptive neuron by K^+ efflux and, consequently antinociception.

Another substance that may also participate of exercise-induced analgesia is irisin. Irisin has been described as a peptide hormone derived from skeletal muscles and cleaved from fibronectin type III domain containing 5 (FNDC5) in response to an induction by peroxisome proliferator-activated receptor γ (PPARγ coactivator 1α (PGC1-α) caused by exercise (119). In addition, as FNDC5 have been shown to induce the expression BDNF in the mice hippocampus in an exercise-dependent manner and irisin also is cleaved FNDC5 (120), we suggesting that this peptide may be involved in the exercise-induced analgesia. Although, a study did not find an association between exercise-induced myokine irisin and regulation of depression, anxiety and stress in obese women (121).

Several endogenous systems are involved in the analgesic effect induced by exercise. This involvement has been found under different intensities, durations, frequencies and modalities of exercise. Furthermore, despite several studies evaluating the participation of one or two endogenous systems in each exercise model used, evidence suggested that these systems may be synergistically activated and that this may occur during exercise (*Fig. 1*). A study showed that the analgesia produced by endogenous opioids may be achieved through the NO/$_c$GMP pathway (122). Furthermore, NO promotes the release of serotonin, an important neurotransmitter involved in the inhibition of nociceptive impulses in the dorsal horn of the spinal cord (123). In addition, a study demonstrated that cannabinoid CB$_1$ and CB$_2$ receptors agonists reduced mechanical allodynia and thermal hyperalgesia by NO/GMPc/K$^+_{ATP}$ pathway activation (124, 125). The NO/cGMP pathway also may be activated by α2-ARs. This activation was demonstrated in a study that found that the analgesia induced by xylazine, a α2-AR agonist, was reversed by NOARG (NOS inhibitor) preadministration and ODQ (cGMP inhibitor) (126). The endocannabinoid system also may activate noradrenergic neurons. This evidence was demonstrated by pretreatment with yohimbine, a α-adrenergic antagonist, which inhibited the analgesic effect induced by delta-*9*-tetrahydrocannabinol (Δ9-THC), the principal psychoactive constituent of *Cannabis* (127).

CONCLUSIONS AND FUTURE DIRECTIONS

In summary, the present review showed that a range of substances may participate in exercise-induced analgesia. Furthermore, future studies are necessary to help unravel other possible endogenous systems involved in exercise-induced analgesia and further elucidate its effects. Furthermore, knowing that exercise releases analgesic substances, investigating the intensity and modality of exercise and at what fitness level analgesia occurs may help devise effective strategies for the use of physical exercise in the treatment of different types of pain, which could ultimately lead to reduced need for pharmacological treatment.

Abbreviations: 2-AG, 2 arachidonoylglycerol; 5HIAA, metabolite 5-hydroxindolacetic acid; α2-ARs, α2-adrenergic receptors; AEA, anandamide; CNS, central nervous system; DLF, dorsal part of the lateral funiculus; DRG, dorsal root ganglion; eNOS, endothelial nitric oxide synthase; FAAH, enzyme fatty-acid amide hydrolase; HIIT, high-intensity interval training; i.c.v., intracerebroventricular; iNOS, inducible nitric oxide synthase; i.p., intraperitoneal; i.pl., intraplantar; i.t., intrathecal; i.v., intravenous; LE, leu-enkephalin; MAPK, mitogen-activated protein kinase; MGL, monoacylglycerol lipase; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; NRM, nucleus raphe magnus; OEA, oleoylethanolamide; PAG, periaqueductal gray matter;

PCPA, ρ-chlorophenylalanine methyl ester; PEA, palmitoylethanolamide; PLC, phospholipase C; PNS, peripheral nervous system; RVM, rostral ventromedial medulla; s.c., subcutaneous; TENS, transcutaneous nerve stimulation; VO$_{2max}$, maximal oxygen uptake;

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