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Potential Serotonergic Interactions With the Anxiolytic-Like Effects of Calcium Channel Antagonists

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EL GANOUNI, S., A. TAZI AND F. HAKKOU. *Potential serotonergic interactions with the anxiolytic-like effects of calcium channel antagonists.* PHARMACOL BIOCHEM BEHAV **60**(2)365–369, 1998.—In a series of experiments, we investigated the interaction between the calcium channel antagonist, nifedipine, and the 5-HT_{1A} agonist, ipsapirone. In the first experiment, we demonstrated that nifedipine (20 mg/kg), and to a lesser extent nimodipine (20 mg/kg), exerted an anxiolytic-like effect as did diazepam (5 mg/kg) in an experimental paradigm based on water consumption in a novel environment. In the second experiment, nifedipine (1.25, 2.5, and 5 mg/kg), and in the third experiment, ipsapirone (1.5, 3.0, and 6.0 mg/kg), have been found to exert a dose-dependent effect in the same test. Finally, a small and ineffective dose of ipsapirone (1.5 mg/kg) potentiated the anxiolytic-like effect of various doses of nifedipine. The data obtained are discussed in terms of the potential anxiolytic-like action of calcium channel antagonists and in relation to their electrophysiological effects. Moreover, the interaction between ipsapirone and nifedipine is discussed in terms of the possible involvement of central serotonergic systems in the behavioral effects of the calcium channel antagonists. © 1998 Elsevier Science Inc.

Calcium channel antagonists Nifedipine Nimodipine Anxiolytics 5-HT_{1A} agonists Ipsapirone Novel environment

RECENT findings indicate that calcium channel antagonists (CCAs) play an important functional role in central nervous system (CNS) disorders and are used in the treatment of cerebrovascular spasm, migraine, and ischemia (27). Moreover, clinical evidence has revealed the psychotropic action of these drugs, which are effective for the treatment of patients with psychiatric disorders (13), including mania, anxiety, and panic disorders (6,15). They also show anticonvulsant (10), sedative and analgesic properties (10,13,16,19). Indeed, CCAs can affect behavioral processes and, therefore, should be considered to be psychotropic agents. These drugs possess antidepressant- (5), anxiolytic- (4,17,30) and neuroleptic-like activities (25).

Using animal models of anxiety, several experiments suggested that some CCAs such as dihydropyridines (DHPs) have an anxiolytic-like effect in a four-plate test (4) and in an elevated plus-maze (26). Nifedipine, a DHP calcium channel antagonist, was also found to have anticonflict activity in a Vogel-type conflict test (17) and an anxiolytic-like effect in water consumption test in a novel environment (30).

Multiple behavioral effects of CCAs may be linked to their modulatory action on the CNS by blockade of some Ca^{2+} channels localized on neurons. Moreover, the Ca^{2+} currents through Ca^{2+} channels is in many cases inhibited by neurotransmitters including serotonin (5-HT). Indeed, 5-HT_{1A} receptor activation causes an important reduction in the calcium current accompanied by a marked slowing of the rate of activation (21,22,28).

The purpose of the present series of experiments was to study the anxiolytic-like effects of CCAs such as DHPs, and to assess their interaction with the 5-HT_{1A} agonist, ipsapirone, to study the role of the central serotonergic system in their behavioral effects. This was done by using a water consumption

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test in a novel environment [e.g., (30), a choice based on previous data obtained in our laboratory and indicating its reproductibility and its sensibility to stressful stimuli.

METHOD

Animals

One hundred seventy male Wistar rats (Laboratory of Pharmacology Casablanca), weighing 220–250 g were used. They were housed three per cage and allowed free access to food and water, except when necessary. The housing room was maintained under constant temperature (22 ± 1°C) and lighting conditions (12 L:12 D cycle).

Apparatus

Anxiety was tested using behavioral responses to a novel environment. For this purpose, a Plexiglas Y-maze was used. The three arms (50 cm long, 15 cm wide, 35 cm high) were positioned at 60° to each other. The floor and walls of arms 1 and 2 were black; those of arm 3 were white. A guillotine door could be used to separate arm 3 from the rest of the maze. A bottle could be connected to the end of arm 2 (black) or arm 3 (white). A light bulb (40 W) was placed 40 cm above arm 3.

General Procedure

During the week preceding the experiments rats were submitted daily to handling, weighing, and placebo injections. They were water deprived for 48 h before the experimental session. The experiment consisted of eight 10-min sessions performed 24 h apart. During the first seven daily training sessions (days 1–7), the guillotine door was closed to prevent access to arm 3 (white), and the water bottle was placed in arm 2 (black). Rats were placed individually at the end of arm 1 and allowed to run into arm 2 to drink water. The amount of water consumed was measured by weighing the bottle before and after each session. Previous experiments have shown slight variations in water consumption after the fifth day of training, leading to a "plateau" from day 5 to day 7 of training.

In the test session (day 8), rats were divided into groups matched according to water consumption during the last training session (day 7). They were given the compounds under study or their vehicles. The guillotine door was removed and the water bottle placed in arm 3 (white). This arm was brightly lit by an additional bulb (40 W), placed 40 cm above the floor. Rats were placed in arm 1 and allowed to run the Y maze to drink water in arm 3.

The procedure used resembles in many details the one described in other reports using a two-compartment apparatus, in which an increase in water intake in the novel environment was observed in animals given various anxiolytic drugs (29).

Drugs

Nifedipine and nimodipine (Bayer, Germany) were dissolved in Tween 80 (1%). Ipsapirone (Bayer, Germany) was dissolved in ethanol (0.5%). Diazepam (Roche, Morocco) was obtained in an injectable form (10 mg/2 ml) and was diluted in saline. Control animals received successive vehicle injections according to the same schedule. Preliminary experiments showed no behavioral effect of Tween 80 (1%), ethanol (0.5%), or both vehicles. The drugs studied were injected intraperitoneally 30 min before the test in a volume of 5 ml/kg.

Pharmacological Treatments

In the first experiment, rats received injections of either 1% Tween 80 (5 ml/kg IP), nifedipine (20 mg/kg IP), nimodipine (20 mg/kg IP), or diazepam (5 mg/kg IP).

To assess if the central serotonergic system contributes to the anxiolytic-like effect of DHPs, the 5-HT_{1A} agonist ipsapirone was studied alone and in combination with several doses of the DHP antagonist, nifedipine.

In the second and third experiments, various doses of nifedipine (1.25, 2.5, or 5 mg/kg) or various doses of ipsapirone (1.5, 3, or 6 mg/kg) were respectively injected to rats. Control animals received vehicles of these drugs.

In the fourth experiment, the 5-HT_{1A} agonist, ipsapirone, was given at the dose of 1.5 mg/kg with nifedipine at the doses of 1.25, 2.5, or 5 mg/kg.

All the studies were conducted according to the "blind-observer" method where one experimenter injected the rats while another performed the tests.

Statistical Analysis

The data obtained were analyzed using a one-way analysis of variance, and post hoc pair-wise comparisons of the group means were performed using the Newman–Keuls test.

RESULTS

In the first three experiments, four groups of 10 rats each were used. They were divided into groups to receive either nifedipine, nimodipine, diazepam, ipsapirone, or their vehicles.

As shown in Fig. 1, in the test session (day 8), control animals drank significantly less water (2.16 ± 0.73 ml) than the mean amount of water drunk during the last training session (day 7) (8.51 ± 0.25 ml) ($p < 0.01$).

Analysis of the mean water consumption in the four experimental groups during the test session showed a significant group effect, $F(3, 36) = 8.32$, $p < 0.001$. Post hoc comparisons of group means indicated a significant increase in water consumption in rats given nifedipine (20 mg/kg), nimodipine (20

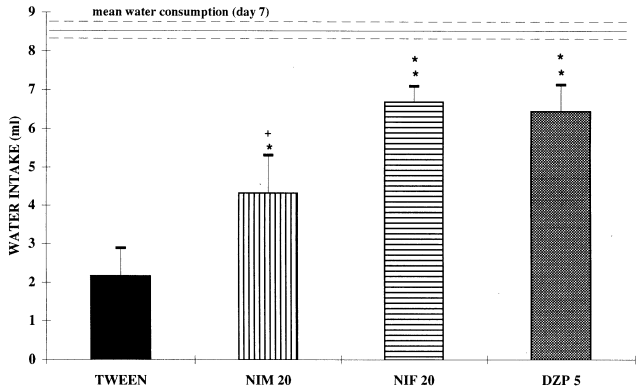

FIG. 1. Water consumption (ml) during the test session in the unfamiliar (white) arm of the Y-maze. Animals were injected 30 min before the session with either Tween 80 (1%) (TWEEN), nimodipine (20 mg/kg) (NIM), nifedipine (20 mg/kg) (NIF), or diazepam (5 mg/kg) (DZP). Each column represents the mean (±SEM) amount of water (ml) drunk. The horizontal bar represents the mean (±SEM) amount of water drunk by all the rats during the last training session (day 7). *$p < 0.05$, **$p < 0.01$ compared to control animals. +$p < 0.05$ compared to diazepam and nifedipine animals ($n = 10$).

mg/kg), and diazepam (5 mg/kg) ($p < 0.01$) compared to control animals.

Figure 2 shows that control animals exhibited a significant decrease in water consumption during the test session compared to the last training day (2.02 ± 0.99 ml vs. 8.81 ± 0.15 ml) ($p < 0.01$). Analysis of variance on mean water consumption during the test session indicated a significant group effect, $F(3, 36) = 3.88$, $p < 0.05$. Pair-wise comparisons indicated that only nifedipine (5 mg/kg) increased water consumption ($p < 0.05$) compared to control animals.

As shown in Fig. 3, control animals exhibited a significant decrease in water consumption during the test session (day 8) (2.93 ± 0.67 ml) compared to the last training day (day 7) (8.25 ± 0.27 ml) ($p < 0.01$).

One-way analysis of variance on the mean water consumption measured during the testing day indicated a significant group effect, $F(3, 36) = 8.87$, $p < 0.001$. Pair-wise comparison of the group means indicated that this was due to a significant increase in water consumption in both ipsapirone (3 mg/kg) and ipsapirone (6 mg/kg) groups compared to the control group ($p < 0.01$).

As indicated in the fourth experiment (Fig. 4), control animals exhibited a significant decrease in water consumption (2.75 ± 0.51 ml) compared to the last training day (8.41 ± 0.18 ml) ($p < 0.01$).

The analysis of variance indicated a significant treatment effect, $F(4. 45) = 31.38$, $p < 0.001$. Post hoc comparison of the group means indicated that ipsapirone (1.5 mg/kg) + placebo did not change water consumption compared to control animals ($p > 0.05$). There was, however, a significant increase in water consumption in ipsapirone + nifedipine (1.25 mg/kg), ipsapirone + nifedipine (2.5 mg/kg), and ipsapirone + nifedipine (5 mg/kg) animals compared to ipsapirone + placebo ($p < 0.01$) and control animals ($p < 0.01$).

DISCUSSION

The present data show that the DHP antagonists, nifedipine or nimodipine alone, increased water consumption in a novel environment, suggesting an anxiolytic-like effect. Indeed, increased food or water consumption in an unfamiliar environment is considered as an index of hypoanxiety (29). These observations confirm previous data reporting an anxiolytic-like effect of nifedipine (30). In accordance with previous results using animal models of anxiety, DHPs has been shown to exert anxiolytic-like effects in an elevated plus-maze (26) in a four-plate test (4), and in a Vogel-type conflict test (17). Also, DHPs were effective against ethanol or diazepam withdrawal-induced anxiety in the plus-maze (26).

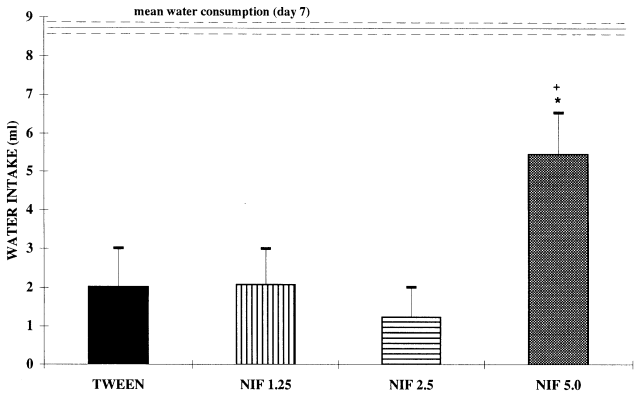

FIG. 2. Water consumption (ml) during the test session in the unfamiliar (white) arm of the Y-maze. Animals were injected 30 min before the session with either Tween 80 (1%) (TWEEN), nifedipine (1.25 mg/kg) (NIF 1.25), nifedipine (2.5 mg/kg) (NIF 2.5), or nifedipine (5.0 mg/kg) (NIF 5.0). Each column represents the mean (±SEM) amount of water (ml) drunk by 10 rats. The horizontal bar represents the mean (±SEM) amount of water drunk by all the rats during the last training session (day 7). *$p < 0.05$ compared to control animals. +$p < 0.05$ compared to nifedipine (1.25 mg/kg) and nifedipine (2.5 mg/kg) animals.

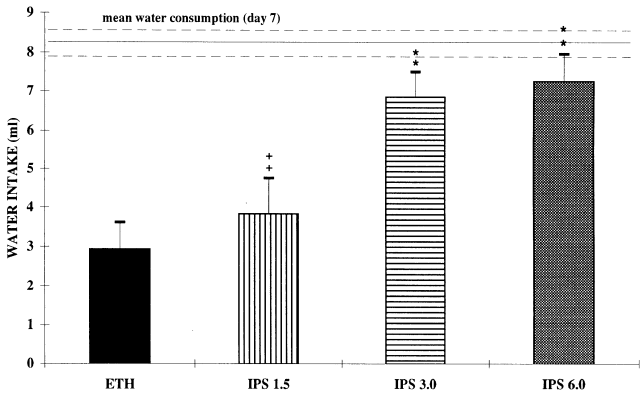

FIG. 3. Water consumption (ml) during the test session in the unfamiliar (white) arm of the Y-maze. Animals were injected 30 min before the session with either ethanol (5%) (ETH), ipsapirone (1.5 mg/kg) (IPS 1.5), ipsapirone (3.0 mg/kg) (IPS 3.0), or ipsapirone (6.0 mg/kg) (IPS 6.0). Each column represents the mean (±SEM) amount of water (ml) drunk by 10 rats. The horizontal bar represents the mean (±SEM) amount of water drunk by all the rats during the last training session (day 7). *$p < 0.05$, **$p < 0.01$ compared to control animals. +$p < 0.05$, ++$p < 0.01$ compared to ipsapirone (3.0 mg/kg), and ipsapirone (6.0 mg/kg) animals.

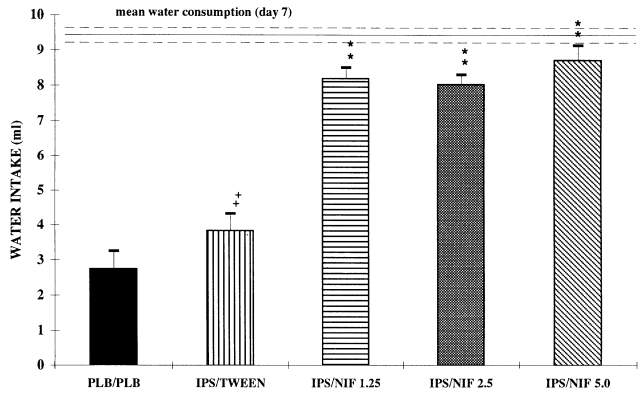

FIG. 4. Water consumption (ml) during the test session in the unfamiliar (white) arm of the Y-maze. Animals were injected 30 min before the session with the two drug vehicles (ETH/TWEEN), ipsapirone (1.5 mg/kg) and nifedipine vehicle (IPS/TWEEN), ipsapirone (1.5 mg/kg) and nifedipine (1.25 mg/kg) (IPS/NIF 1.25), ipsapirone (1.5 mg/kg) and nifedipine (2.5 mg/kg) (IPS/NIF 2.5), or ipsapirone (1.5 mg/kg) and nifedipine (5.0 mg/kg) (IPS/NIF 5.0). Each column represents the mean (±SEM) amount of water (ml) drank by 10 rats. The horizontal bar represents the mean (±SEM) amount of water drunk by all the rats during the last training session (day 7). *$p < 0.05$, **$p < 0.01$ compared to control (PLB/PLB) animals. ++$p < 0.01$ compared to IPS/NIF 1.25, IPS/NIF 2.5, and IPS/NIF 5.0 animals.

However, there is experimental evidence indicating a lack of anxiolytic-like activity for DHPs using social interaction, plus-maze (7,8), open-field (24), and holeboard tests (31).

Nevertheless, it has been suggested that the anxiolytic-like effect of certain DHPs is observed when a high degree of behavioral inhibition, presumably due to increased fear, is present (24). This condition is probably represented in our experimental test combined with an unfamiliar environment.

In the present study, nifedipine was shown to be as effective as diazepam and induced a dose-dependent increase in water consumption in a novel environment, with an effective dose of 5.0 mg/kg. Consistent with previous results (30), lower doses of nifedipine were ineffective.

The mechanism by which nifedipine and nimodipine mediate behavioral effects is still unknown. However, it is clear that these compounds are able to pass the blood–brain barrier, and reach higher levels in the CNS, which are closely related to emotional processes, such as the limbic system (3). From the biochemical data available, it has been shown that DHPs could have a defined functional role in the brain, in the control of neurotransmitter release such as acetylcholine (Ach) (20) serotonin (5-HT) (18), dopamine (DA) (14), or noradrenaline (23). Obviously, Ca^{2+} plays an important role in the regulation of release of neurotransmitters from nerve terminals and dendrites (32), and this regulation is mediated by Ca^{2+} channels that are blocked by several CCAs. Moreover, the behavioral changes in rats after chronic administration of DHPs confirm the possibility that Ca^{2+} channels have neuromodulatory or regulatory actions on the CNS (1,16). In light of these above observations, one hypothesis suggests that anxiolytic-like effects of DHPs may be related to their modulatory effect on the central serotonergic system (2).

Our data show that ipsapirone dose dependently increased water consumption in a novel environment, suggesting an anxiolytic-like effect. The doses used in the present study show that ipsapirone is active at the doses of 3 and 6 mg/kg. The combined administration of an ineffective dose of ipsapirone (1.5 mg/kg) with various doses of nifedipine potentiates the anxiolytic-like effects of ipsapirone. These results extend our previous data showing a potentiation of the anxiolytic-like effects of an ineffective dose of nifedipine by various doses of ipsapirone (30).

Activation of 5-HT_{1A} receptors in the dorsal raphe (DR) neurons by 5-HT or by 8-OH-DPAT slowed the rate of Ca^{2+} currents by inhibition or blockade of Ca^{2+} channels localized on dendrites and cell bodies via G-proteins (21), and therefore reduce the release of neurotransmitters in nerve terminals. This inhibitory effect of 5-HT also has been performed in rat ventromedial hypothalamic neurons (28), and in neocortical pyramidal neurons (9). Recent electrophysiological data suggest that DHPs interact with 5-HT receptor subtypes, which may partially explain their anxiolytic-like effect. For instance, all DHP antagonists directly inhibit 5-HT_3 receptors (12), which are reported to mediate some anxiolytic-like effects (11). These findings argue that 5-HT receptor agents modulate Ca^{2+} channels, and vice versa.

In light of the above data, combined with these interpretations, activation of 5-HT_{1A} by ipsapirone could indirectly increase Ca^{2+} channels sensitivity to nifedipine.

However, previous work using both in vivo and in vitro experiments suggest that the inhibitory action of DHPs on neurotransmitter release occurs in a voltage- and time-dependent manner, when channels are previously affected by pharmacological manipulation or by the presence of a pathologic factor such as ischemic damage (18). Ca^{2+} channel subunits of some neuronal cells could be altered in such a way that they would be sensitive to DHP antagonists.

In conclusion, it is suggested that the anxiolytic-like effects of DHPs are mediated by neurobiological substrates directly involved in the pathogeny of anxiety (i.e., the central serotonergic system). This hypothesis is over simplified when compared to the quite complex pharmacological properties of the central serotonergic system and its interaction with DHPs. Further experiments using biochemical assays are necessary to confirm this hypothesis.

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