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Effect of clonidine on ultrasonic vocalization in preweaning rats

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Summary. The present study was undertaken to investigate the involvement of the noradrenergic neurotransmission system in the ultra sonic callings emitted by rat pups separated from their mother and exposed to cold stimulation. The investigation was primarily performed by help of agents selectively affecting the α-adrenoceptors: the α_2-agonist clonidine, the α_1-antagonist prazosin and the α_2-antagonist idazoxan.

Clonidine dose-dependently stimulated the amount of ultra sonic vocalization, an effect not solely dependent upon the effect of clonidine on body temperature. In a developmental study it was found that clonidine uniformly stimulated crying at all ages from 4 days of age up to 18 days of age, that is during the whole preweaning period. Clonidine stimulated ultrasonic crying in rat pups, devoid of presynaptic catecholamine (CA) neurons by combined pretreatment with the monoamine depletor, reserpine, and the inhibitor of CA-synthesis, α-methyl-tyrosine. This finding suggested that the stimulating effect of clonidine on ultrasonic vocalization was mediated by postsynaptic adrenoceptors.

In pups, 12 days of age, idazoxan blocked the effect of cold stimulation on ultra sonic crying, suggesting that α_2-adrenoceptors, presumably postsynaptic ones, mediated this kind of stimulation. Idazoxan also antagonized the effect of clonidine, but only at a dose effective also in control pups. Prazosin had no effect on cold-stimulated crying, but antagonized the effect of clonidine, suggesting that the effect of clonidine was also mediated by α_1-receptors. At 18 days of age, prazosin no longer antagonized the effect of clonidine, whereas the antagonizing action of idazoxan was reinforced.

The age-dependent variation in responsiveness to the adrenergic drugs suggest maturational changes in the function of the CA-system occurring between 12–16 days of age.

Keywords: Ultrasonic vocalization, rat pups, clonidine, noradrenaline, adrenoceptors.

Introduction

A rat pup separated from its nest, mother and littermates emits callings in the ultrasonic frequency range. The cries call the attention of the mother, making her search for and eventually retrieve the pup to the nest (Allin and Banks, 1972). The amount of ultrasonic vocalization varies with the age of the pup, displaying a characteristic developmental pattern (Noirot, 1966; Hård et al., 1982). A neonatal pup, unable to leave its nest, emits very few callings. From about one week of age, when the pup is able to crawl around (Blanck et al., 1967), the number of cries increases up to a maximal level at 9–11 days of age. Thereafter, the amount of callings decreases to subside at about 16 days of age. At that age several other developmental landmarks are reached: a functional thermoregulation (Adolph, 1957), opening of the eye-lids, mastering of turning around during free fall in the air (Hård and Larsson, 1975).

Ultrasonic vocalization may be blocked by agents with anxiolytic actions like diazepam and ethanol even at doses not inducing sedation (Gardner, 1985a; Insel et al., 1986; Sales et al., 1986; Engel and Hård, 1987). The sensitivity to anxiolytic drugs, combined with its ethological significance, renders ultrasonic vocalization a suitable tool for studies on the role of neurochemical systems involved in fear/anxiety reactions occurring during preweaning age.

Previous studies suggested an important role of the serotonin system (5-HT) in this behavior. Neonatal i.c.v. administration of the serotonergic neurotoxin 5,7-dihydroxytryptamine strongly suppressed ultrasonic vocalization during the whole development eliminating the maximal response amplitude at about 10 days of age (Hård et al., 1982). Similarly, subchronic treatment with para-chloro-phenylanaline, preferentially depleting 5-HT, decreased the amount of crying. In this case the vocalization could be reinstalled by subsequent treatment with the 5-HT precursor 5-hydroxytryptophan (Hård et al., 1982; Gardner, 1985).

Many studies have suggested an important role for noradrenaline (NA) in anxiety reactions (see Redmond, 1977). In spite of this we found no effect on ultrasonic vocalization of neonatal treatment with the catecholaminergic neurotoxins 6-hydroxy-dopamine (6-OHDA) (Hård et al., 1982) or DSP4 (Hård and Engel, unpublished). At the doses used in the experiment with 6-OHDA, the treatment selectively reduced whole brain levels of NA by about 50%, thus leaving an appreciable number of functional NA neurons. As these neurones may be sufficient for a normal expression of vocalization the participation of NA in this behavior can not be ruled out.

A further examination of the role of NA in distress crying was therefore undertaken, using α-adrenoceptor active drugs as tools. Previous studies have indicated that the α_2-adrenoceptor agonist clonidine relieves feelings of anxiety or withdrawal symptoms during abstinence from morphine- or alcohol-dependence in human and animals (Björkqvist, 1975; Tseng et al., 1975; Aghajanian, 1978; Gold et al., 1978; Kostowski and Trzaskowska, 1980;

Wålinder et al., 1981; Wilkins et al., 1983). There are also reports suggesting an ameliorating effect of clonidine on anxiety disorders (Svensson et al., 1978; Hoehn-Saric et al., 1981; Liebowitz, 1981). The anxiolytic effects of clonidine are thought to be mediated by an agonistic action on presynaptic α_2-receptors causing an inhibition of overactive NA-neurons (Redmond 1977; Aghajanian and VanderMaelen, 1982). The rationale for this notion was afforded by electrophysiological studies indicating inhibition of NA neurons in locus coeruleus induced by the effects of clonidine on presynaptic α_2-adrenoceptors (Svensson et al., 1975; Aghajanian and VanderMaelen, 1982).

On the basis of these observations we adopted clonidine as a suitable tool for studies on the possible role of α_2-adrenoceptors on ultrasonic vocalization, conceived as a reaction of distress.

Materials and methods

Animals

The animals belonged to a strain of Wistar rats purchased from Möllegaard Breeding Lab. and bred in our laboratory. The animals were maintained under controlled temperature (22 °C) and humidity (50%) conditions and on a 12 : 12 h dark-light cycle with light off at 10.00 a.m. Food and water were freely available. At birth (Day 0), each litter was culled to eight pups.

Distribution of animals on various treatment groups

If not otherwise stated, the following procedure was applied for the distribution of animals on various treatment groups. In each experiment several litters were used. Within each litter, two rats were randomly selected as controls and six as experimental animals with two animals randomly selected for each one of the three doses or combinations of treatments used.

Behavioral testing

Before drug treatment the whole litter was removed from the mother and placed in a plastic cage immersed in a water bath (37 °C), where it was left undisturbed for at least 15 minutes. After drug treatment the pups were returned to the litter. At the time of testing the rat was placed in a circular jar (diameter: 11 cm) fixed in a bath containing ice and water giving a temperature of +2 °C–+4 °C in the interior of the jar. The bath and the jar were enclosed in a sound proof test chamber, illuminated by a 15 W white light bulb.

The vocalizations of the pup were picked up by a microphone for the detection of ultrasonic callings (BT 1795, Knowles Electronics Inc. with flat response in the frequency range 20–100 kHz). The microphone signals were fed through a preamplifier, connected with an electronic counter. The counter displayed the cumulative duration of all callings emitted during the testing period (5 min) by help of an electronic gate and a stable 1 kHz generator.

To calm down the rat pup before being replaced to its littermates after testing, the pup was placed alone for 10 min in a separate cage, placed in a 37 °C water bath.

Body temperature

Body temperature was measured by inserting a probe containing a thermo-couple in the nape of the neck.

Statistics

In order to minimize the influence of inter-litter variability the statistical analyses were performed in the following way. For the comparison between animals subjected to two different treatments, the behavioral observations within each litter were ranked and treated according to the Mann-Whitney U-test procedure (Bradley, 1968). The results from the various litters were pooled into one test by adding the U-test-variables in main accordance with the procedure used in Mantel's test (Mantel, 1963). In the present procedure, however, a more accurate approximation of the distribution of the test variable was achieved by using the Edgeworth expansion (Cramér, 1946). Two-tailed levels of significance were consistently used.

Experiments

1 A Effects of various doses of clonidine on ultrasonic vocalization in rat pups 12 days of age

Procedure

Clonidine was given in following doses: 0.005, 0.01, 0.02, 0.025, 0.1, and 0.4 mg/kg body weight. The drug was dissolved in 0.9% saline and injected s.c. 20 min before testing. The controls were treated with saline only.

Results and discussion

As shown in Fig. 1 clonidine increased the amount of ultrasonic vocalization in a dose-dependent fashion. During the 5 min exposure to the cold environment,

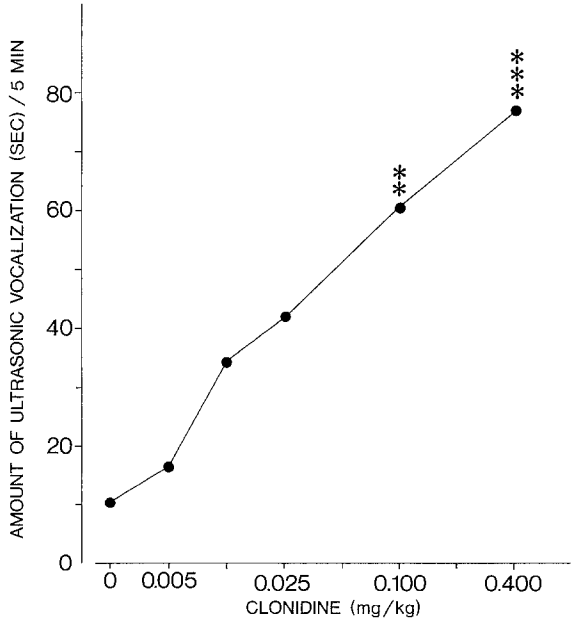

Fig. 1. The effects of various doses of clonidine on the amount of ultrasonic vocalization emitted by rat pups, 12 days of age, exposed to an ambient temperature of 4 °C for 5 min. 20 min prior to test the animals were s.c. treated with the dose of clonidine indicated or with the vehicle. Shown are median values for 8–10 animals tested at each dose. *** $p < 0.001$; ** $p < 0.01$

the body temperature of the control animals was decreasing, signifiying the incomplete thermoregulation in rats of this age (Adolph, 1957). The decrease of body temperature was further accelerated by increasing doses of clonidine in accordance with previous observations (Reinstein and Isaacson, 1981). As indicated by the controls, exposure to a cold environment induces both ultrasonic vocalization and lowering of the body temperature.

If ultrasonic vocalization depends not only on stimulation of peripheral thermoreceptors but also on central effects of lowered body temperature, the effects of clonidine on the vocalization might be mediated by its influence on body temperature. This possibility was explored in two experiments studying the effects of clonidine on ultrasonic vocalization elicited by conditions not involving cold stimulation.

1B Effects of clonidine on ultrasonic vocalization in rat pups exposed to a high ambient temperature

Procedure

The same general procedure as in experiment 1A was applied with the exception that the interior temperature of the testing cage was raised to 38 °C or 40 °C by immersion of the cage in a hot water bath.

The pups, 12 days of age, were treated with 0.025, 0.100, and 0.4 mg/kg clonidine in accordance with the procedure in experiment 1A.

Results

As evidenced by Fig. 2, control rat pups emitted very few ultrasonic cries when placed in the warm environment after separation from their mother. In spite of this, treatment with clonidine elicited appreciable amounts of crying at all the doses used and under both conditions of ambient temperature. The body temperature decreased at the higher doses of clonidine. At an ambient temperature of 40 °C the body temperature was similar, 38.1 °C, in both controls and those experimentals treated with 0.025 mg/kg clonidine. In spite of this the ultra sonic vocalization was increased in the drug treated pups. At an ambient temperature of 38 °C the body temperature of the controls was 37.9 °C, which is lower than the one reached by the 0.025 mg clonidine treated pups at an ambient temperature at 40 °C. In spite of this the control pups vocalized far less than the clonidine pups so treated. The results of the present experiment thus indicate that clonidine may increase the amount of ultra sonic vocalization independent of changes in body temperature.

1C Ultra sonic vocalization in pups freely hanging down

Procedure

In accordance with the general procedure the rat pups were placed for at least 15 min in a cage immersed in a 37 °C water bath and s.c. treated with 0.025,

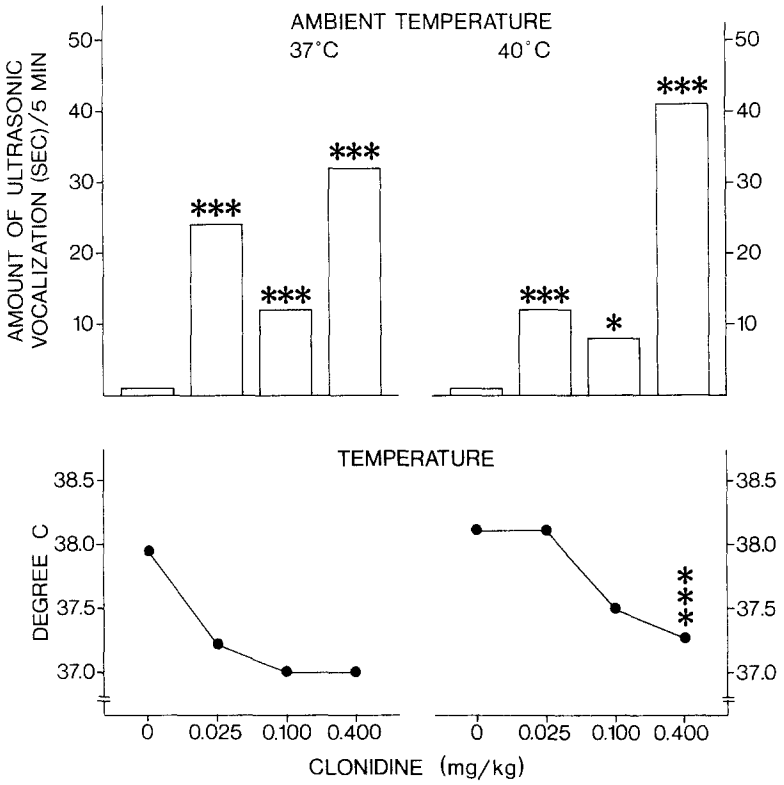

Fig. 2. The effects of various doses of clonidine on body temperature and on the amount of ultrasonic vocalization emitted by rat pups, 12 days of age, exposed to an ambient temperature of 38 °C or 40 °C for 5 min. 20 min prior to test the animals were s.c. treated with the dose of clonidine indicated or with the vehicle. Shown are median values for 10 animals tested at each dose. *** $p < 0.001$; * $p < 0.05$

0.100, and 0.400 mg/kg clonidine 20 min before test. At test, the rat pup was lifted by its tail and held in a hanging position with its head in front of the microphone. The ultrasonic vocalizations were recorded for 1 min.

Results

As indicated in Fig. 3, clonidine dose-dependently increased the amount of vocalization in rat pups, whose crying was stimulated by hanging helpless in the air.

Conclusions

The rationale for the present experiments was the observation that clonidine may attenuate feelings of distress in patients with anxiety disorders or suffering from abstinence from morphine or alcohol. As ultrasonic vocalization in the rat pup is considered a distress response, it was expected that treatment with clonidine should decrease the amount of vocalization. Contrary to this expectation clonidine increased distress crying, an effect which could not be solely attributed to the effect of clonidine on body temperature.

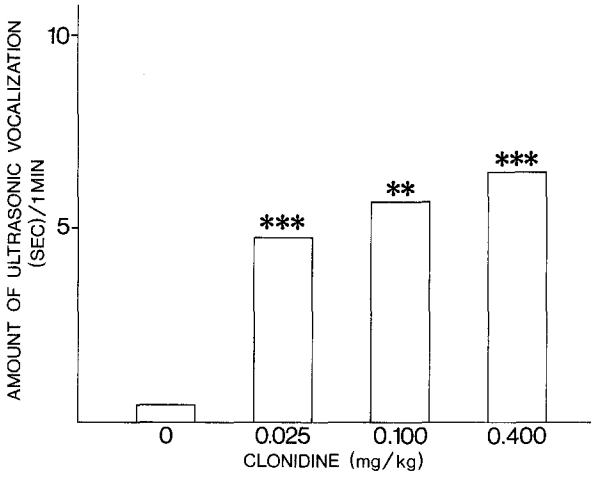

Fig. 3. The effects of various doses of clonidine on the amount of ultrasonic vocalization emitted by rat pups, 12 days of age, held by their tails in a head-down hanging position for 1 min. 20 min prior to test the pups were s.c. treated with the dose of clonidine indicated or with the vehicle. Shown are median values for 10 animals tested at each dose. *** $p < 0.001$; ** $p < 0.01$

One reason for the apparently contradictory results might be that clonidine during early ages exerts effects dissimilar from those at adult ages. Support for this possibility is provided by reports on the effects of clonidine on motor activity in preweaning rats (Kellogg and Lundborg, 1972; Reinstein and Isaacson 1977, 1981; Nomura and Segawa, 1979; Nomura et al., 1980; Pappas and Walsh, 1983). In contrast to the sedative effects repeatedly observed in adult rats (Laverty and Taylor, 1969; Maj et al., 1972; Strömbom, 1975) clonidine stimulated motor activity in rat pups up to about 14 days of age (Reinstein and Isaacson, 1977; Nomura, 1980). This age-dependent shift of effect is considered to reflect maturational changes in the α_2-adrenoceptor-population normally affected by clonidine. We therefore decided to investigate if clonidine exerted similar age-dependent effects on ultra sonic vocalization. This was performed by a study covering the whole age-span from birth to weaning.

2 The effect of clonidine on ultrasonic vocalization during different stages of ontogenetic development

As mentioned in the Introduction the ontogeny of ultra sonic vocalization in the rat displays a characteristic developmental pattern. From a low level of vocalization during the first days after birth, the amount of crying rapidly increases from 6 days of age to reach a peak level at 10–11 days of age. After that age the amount of vocalization subsides to eventually disappear at about 16–18 days of age.

In the present experiment we studied the effect of clonidine during the whole development from 2 days of age up to an age of 22 days, when ultra sonic crying normally is absent.

Procedure

The effect of clonidine on ultra sonic vocalizaton was studied in pups of the following ages in days: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22. Experimentally naive pups were used at each age. The number of litters tested at each age varied between 5 to 7 litters. Maintenance, drug treatments and testing were performed in accordance with the general procedure including an ambient temperature of 4 °C in the testing cage. Twenty min before test, the pups were treated s.c. with 0.025, 0.100 or 0.400 mg/kg clonidine, while the controls were treated with the vehicle.

Results

As shown in Fig. 4 treatment with clonidine increased the amount of ultra sonic vocalization at each age from day 6 up to day 8 of age. In Fig. 5 the longitudinal development of the effect on crying of the highest dose of clonidine used (0.400 mg/kg) is compared to the normal development of crying. Clonidine exerted no effects on crying at day 2, and only a slight increase with the highest dose at days 4. After day 20 the drug could not any longer elicit ultra sonic vocalization.

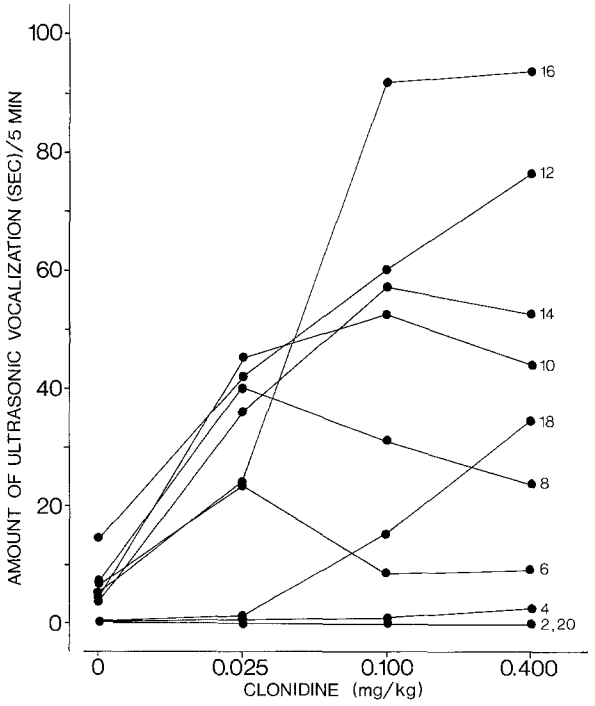

Fig. 4. The effects of various doses of clonidine on the amount of ultrasonic vocalization emitted by rat pups of different ages from day 2 up to day 20. The ages are indicated by the numbers in the right part of the figure. At each age 5–7 litters of animals were tested, each litter containing 8 pups. 20 min prior to test the animals were s.c. treated with the dose of clonidine indicated or with the vehicle

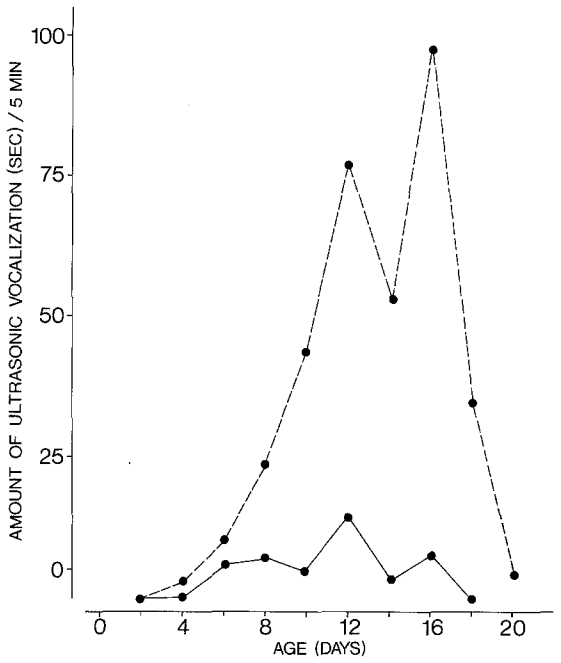

Fig. 5. The effects of 0.400 mg/kg clonidine (● – – – ●) compared to vehicle (●———●) on the amount of ultra sonic vocalization emitted by rat pups of different ages from day 2 up to day 20. The animals were treated s.c. 20 min prior to test

From day 12 onwards the effect of clonidine on the vocalization increased dose-dependently. During the age interval 6–10 days the effect reached its highest level already at the lowest dose (0.025 mg/kg).

Conclusion

Previous developmental studies on the effect of clonidine on motor activity indicated a transition of effect from a stimulating action before 14 days of age to a sedating one after that age (Reinstein and Isaacson, 1977, 1981; Nomura and Segawa, 1979). By contrast, clonidine exerted only a uniformly excitatory action on ultra sonic crying during the whole age period from day 6 to 8. Clonidine exerted no effect during the immediate neonatal period. On the other hand, clonidine elicited appreciable amounts of crying during the normally silent preweaning stage from day 16 onwards. However, after the beginning of the weaning period at day 21, clonidine could not any longer elicit ultra sonic vocalization.

The results of experiments 1 and 2 thus show that clonidine, only exerts a stimulatory action on ultra sonic vocalization. The problem of the neurochemical mediation of this effect was addressed in the following experiments.

3 Post- or pre-synaptic mediation of the effect of clonidine on ultra sonic vocalization

The following series of experiments were performed to establish the type of α-adrenergic receptors mediating the effect of clonidine on ultra sonic vocalization.

In the first experiment we investigated if the effect of clonidine was mediated by α-adrenoceptors located pre- or post-synaptically.

For this purpose the influence of the presynaptic NA-neurons was pharmacologically eliminated by the combined treatment with the monoamine-depleting agent, reserpine, and the inhibitor of CA-synthesis, α-methyl-tyrosine.

Procedure

16 rat pups, 12 days of age, were maintained and behaviorally tested according to the general procedure at an ambient temperature of 4 °C. 6 hours before test the animals were treated s.c. with 5 mg/kg reserpine (dissolved in 5.5% glucose and a few drops of acetic acid). 4 hours later this treatment was followed by s.c. injection of 200 mg/kg α-methyl-tyrosine (dissolved in 0.9% saline). 20 min before test half of the pups were treated with 0.100 mg/kg clonidine (dissolved in 0.9% saline) and the other half with the vehicle only.

Results

As indicated in Fig. 6 the combined treatment with reserpine and α-methyl-tyrosine strongly decreased the ultra sonic vocalization elicited by the cold environment. Treatment with clonidine restored the amount of vocalization to a level comparable to that normally observed in intact rats treated with clonidine (see Fig. 1).

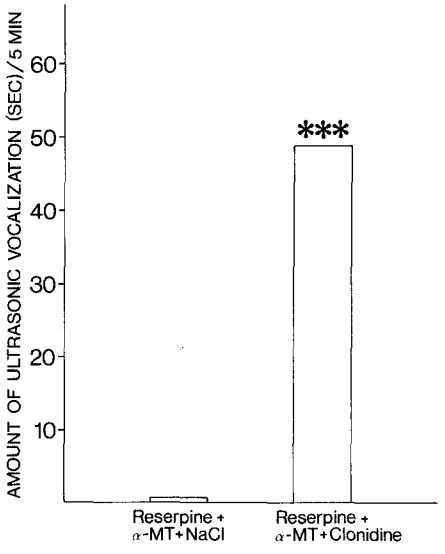

Fig. 6. The effect of clonidine on the amount of ultrasonic vocalization emitted by rat pups, 12 days of age, pretreated with reserpine (5 mg/kg) s.c. 6 hours prior to test, and with α-methyl-tyrosine (200 mg/kg) s.c. 2 hours before test. 20 min prior to test one half of the animals were s.c. treated with clonidine (0.100 mg/kg) and the other half with 0.9% saline. During the 5 min test the pups were exposed to an ambient temperature of 4 °C. Both experimental and control groups contained 8 animals. Shown are median values for the amount of ultra sonic vocalization. *** $p < 0.001$

Conclusion

The present results show that the combined treatment with reserpine and α-methyl-tyrosine nearly extinguished ultra sonic vocalization suggesting an important role for the monoamines in this behavior. As this pretreatment eliminates influences via presynaptic adrenoreceptors the restoration of the crying achieved by treatment with clonidine suggests that the action of clonidine is mediated by postsynaptically located adrenoceptors. The same conclusion was reached in an experiment, not reported, where the presynaptic influence of NA-neurons was eliminated by neonatal treatment with the neurotoxin 6-hydroxydopamine causing a selective lesion on the developing NA-system (compare Hård et al., 1982).

In the following experiment we investigated if these post-synaptically mediated effects involved receptors of α_1- or α_2-type.

4 Effects of α_1- and α_2-adrenoceptor antagonists on clonidine-induced ultra sonic vocalization

A Effects on rat pups 12 days of age

To investigate if the effects of clonidine on ultrasonic crying was mediated by postsynaptic α_1- or α_2-adrenoceptors we tried to antagonize the effects of clonidine by antagonists selective for each type of α-receptor respectively. Blockade of the α_1-adrenoceptor was achieved by the specific α_1-receptor antagonist prazosin (Menkes et al., 1981) and of the α_2-adrenoceptor by the specific α_2-receptor antagonist idazoxan (Dettmar et al., 1983; Doxey et al., 1983; Freedman and Aghajanian, 1984).

Procedure

The experiments were performed in accordance with the general procedure using animals 12 days of age tested at an ambient temperature of 4 °C. To assess the effects of various doses of prazosin pups were s.c. treated 15 min before test with 0.125, 0.25, or 0.50 mg/kg of the drug (dissolved in 5.5% glucose with a few drops of acetic acid). Controls were treated with the vehicle only.

The same assessment of the effects of idazoxan was performed by s.c. treatment 45 min before test with 0.05, 0.10, and 0.50 mg/kg body weight (drug dissolved in distilled water).

In trials to antagonize the effect of clonidine (0.10 mg/kg) prazosin at a dose of 0.50 mg/kg body weight or idazoxan at a dose of 0.10 or 1.0 mg/kg was used. Clonidine was injected 20 min before test, the two other drugs as described above. Control animals were treated according to corresponding time schedules using adequate vehicles.

Results

Fig. 7 shows that prazosin over a wide range of doses did not exert any effects on ultra sonic vocalization. Prazosin antagonized the increase of crying induced by clonidine without exerting an effect of its own (Fig. 8).

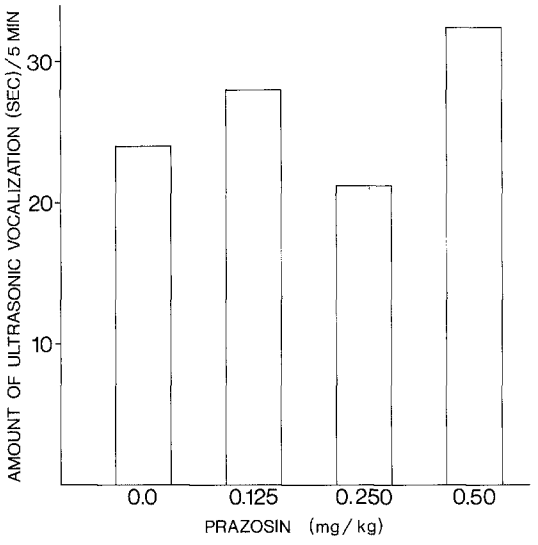

Fig. 7. The effects of various doses of prazosin on the amount of ultrasonic vocalization emitted by rat pups, 12 days of age, exposed to an ambient temperature of 4 °C for 5 min. 15 min prior to test the animals were s.c. treated with the dose of prazosin indicated or with the vehicle. Shown are median values for 12 animals tested at each dose.

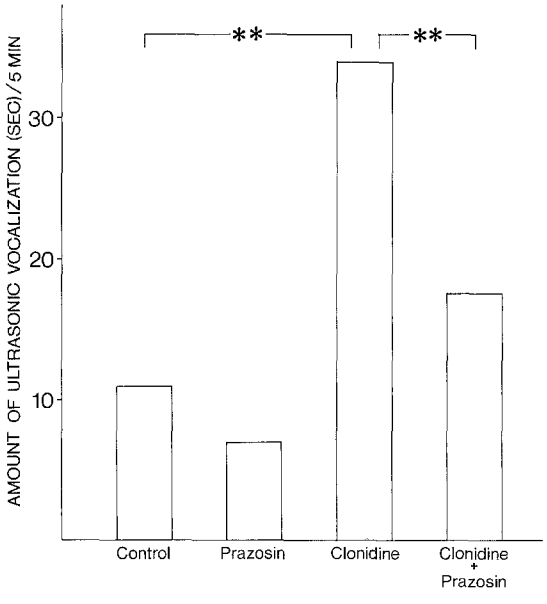

Fig. 8. The effects of prazosin or clonidine alone, or in combination on the amount of ultrasonic vocalization emitted by rat pups, 12 days of age, exposed to an ambient temperature of 4 °C. Prazosin (0.5 mg/kg) or vehicle was s.c. administered 15 min prior to test, whereas clonidine (0.1 mg/mg/kg) or vehicle was s.c. injected 20 min before test. At each combination of treatments 10 animals were used. Shown are median values for the amount of vocalization. ** $p < 0.01$

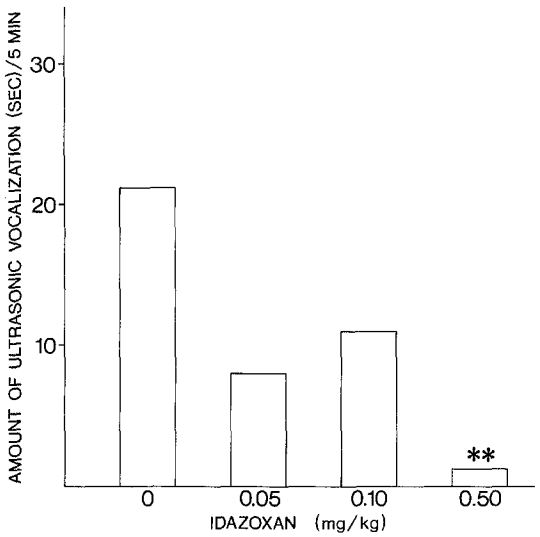

Fig. 9. The effects of various doses of idazoxan on the amount of ultrasonic vocalization emitted by rat pups, 12 days of age, exposed to an ambient temperature of 4 °C for 5 min. 45 min prior to test the animals were s.c. treated with the dose of idazoxan indicated or with the vehicle. Shown are the median values for 12 animals tested at each dose. ** $p < 0.01$

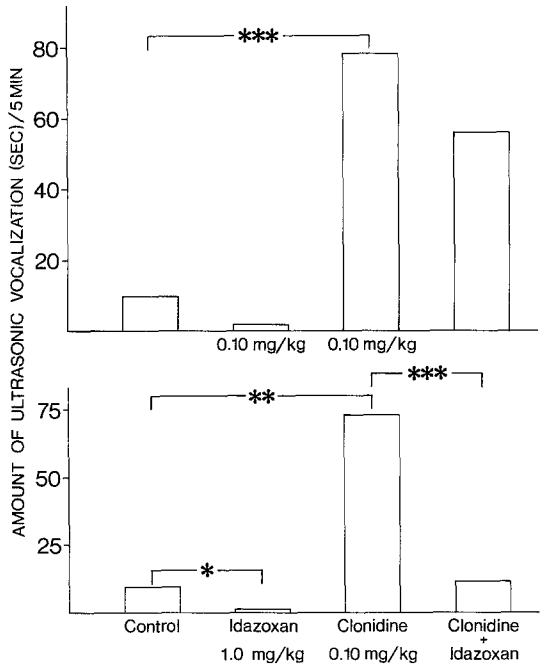

Fig. 10. The effects of idazoxan or clonidine alone, or in combination on the amount of ultrasonic vocalization emitted by rat pups, 12 days of age, exposed to an ambient temperature of 4 °C. Idazoxan (0.1 or 0.5 mg/kg) or vehicle was s.c. administered 45 min prior to test, whereas clonidine (0.1 mg/mg/kg) or vehicle was s.c. injected 20 min before test. At each combination of treatments 12 animals were used. Shown are median values for the amount of ultra sonic vocalization. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

In contrast to prazosin, idazoxan decreased the amount of ultra sonic vocalization in a dose dependent manner as indicated by Fig. 9. Similarly to prazosin, idazoxan antagonized the stimulatory action of clonidine on crying, reaching statistical significance only at the highest dose however (1.0 mg/kg) (Fig. 10).

Conclusion

As expected from the designation of idazoxan as an α_2-adrenoceptor antagonist, this drug blocked the increase of ultra sonic vocalization induced by clonidine, considered as an α_2-receptor agonist. With increasing doses these two drugs exerted opposite actions on crying: clonidine increasing, idazoxan decreasing.

However, in spite of the conception of clonidine as a selective α_2-receptor agonist, prazosin, considered a selective α_1-receptor antagonist, also antagonized the stimulatory action of clonidine.

Therefore, in the present experiments, performed on rats 12 days of age, clonidine seems to exert an action less selective than that observed in other experimental situations. The results may indicate that the α-adrenoceptor population at this age has not yet differentiated into functionally separate α_1- and α_2-receptor populations. This hypothesis was tested in the following experiment using rat pups, 18 days of age. At that age clonidine still exerts a stimulatory effect on ultra sonic vocalization as evidenced by the results of experiment 1.

B Effects on rats 18 days of age

Procedure

Rats, 18 days of age, were treated with clonidine (0.10 mg/kg), prazosin (0.50 mg/kg) and idazoxan (0.10 mg/kg) in accordance to the schedule applied in the preceding experiment 4A.

Results

As indicated in Figs. 11 and 12 and confirming the results in experiment 1, clonidine stimulated ultra sonic vocalization even at day 18, when normally the amount of crying is very low. Like the results obtained on animals 12 days of age, idazoxan antagonized the effect of clonidine in rat pups 18 days of age (Fig. 12). By contrast, the antagonistic action of prazosin on clonidine-induced vocalization, effective at 12 days of age, was absent at day 18 (Fig. 11).

Conclusion

In rat pups tested at 12 or 18 days of age the α_2-receptor antagonist idazoxan antagonized the stimulatory effects of clonidine on ultra sonic vocalization. In contrast, the α_1-receptor antagonist prazosin exterted an antagonistic action only at day 12, exerting no effect at day 18. One possible explanation for this discrepancy is that the α-receptor population mediating the action of clonidine on crying has not yet functionally differentiated into subpopulations of α_1- and α_2-receptors, respectively.

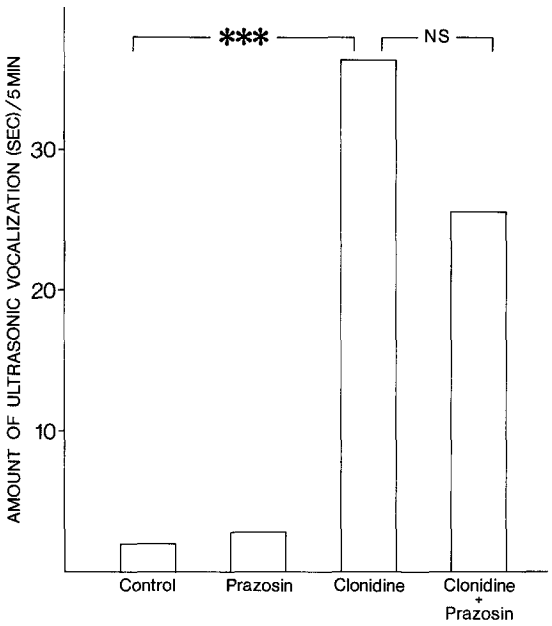

Fig. 11. The effects of prazosin or clonidine alone, or in combination on the amount of ultrasonic vocalization emitted by rat pups, 18 days of age, exposed to an ambient temperature of 4 °C. Prazosin (0.5 mg/kg) or vehicle was s.c. administered 15 min prior to test, whereas clonidine (0.1 mg/mg/kg) or vehicle was s.c. injected 20 min before test. At each combination of treatments 14 animals were used. *** $p < 0.001$; *NS* not significant

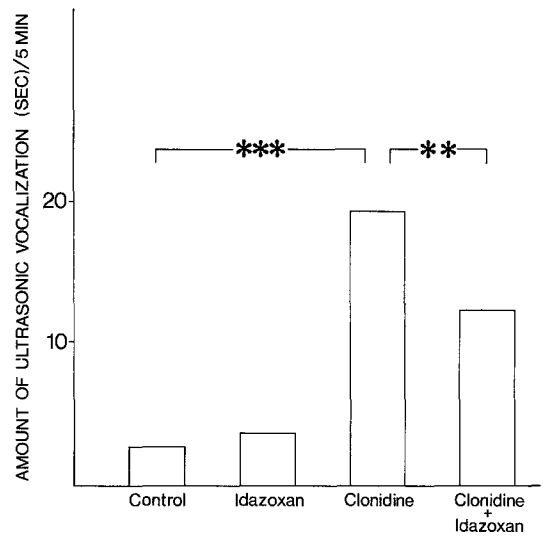

Fig. 12. The effects of idazoxan or clonidine alone, or in combination on the amount of ultrasonic vocalization emitted by rat pups, 18 days of age, exposed to an ambient temperature of 4 °C. Idazoxan (0.1 mg/kg) or vehicle was s.c. administered 45 min prior to test, whereas clonidine (0.1 mg/mg/kg) or vehicle was s.c. injected 20 min before test. At each combination of treatments 14 animals were used. Shown are median values for the amount of vocalization. *** $p < 0.001$; ** $p < 0.01$

C Effects on rats 16 days of age

To estimate more closely the age for the disappearance of the antagonistic action of prazosin on clonidine-induced crying, rat pups 16 days of age were also investigated. The experiment was performed with the same doses of prazosin and clonidine and according to the same schedule as in the preceding experiments 4A and 4B.

The results, shown in Fig. 13, indicate that prazosin neither at day 16 antagonized the effect of clonidine. This indicates that the disappearance of the capacity of prazosin to antagonize the stimulating action of clonidine on crying is occurring at some age between 12 and 16 days of age.

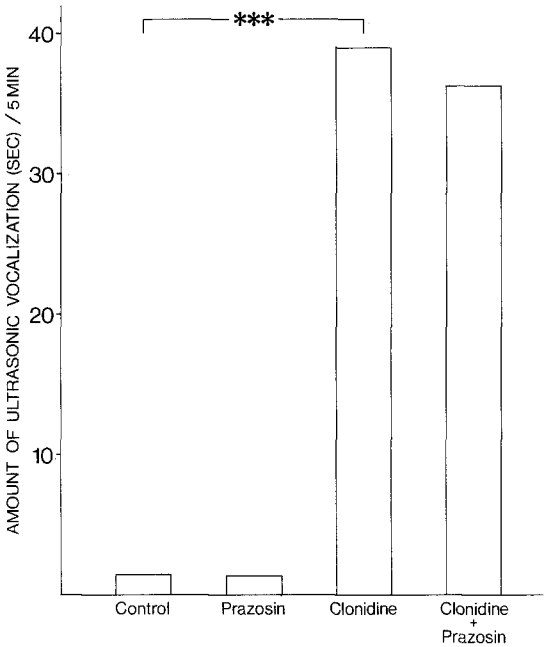

Fig. 13. The effect of prazosin or clonidine alone, or in combination, on the amount of ultrasonic vocalization emitted by rat pups, 16 days of age, exposed to an ambient temperature of 4 °C. Prazosin (0.5 mg/kg) or vehicle was s.c. administered 15 min prior to test, whereas clonidine (0.1 mg/mg/kg) or vehicle was s.c. injected 20 min before test. At each combination of treatments 14 animals were used. Shown are median values for the amount of ultra sonic vocalization. *** $p < 0.001$

General discussion

The possible role of the noradrenergic transmission system in ultra sonic vocalization was previously studied in rat pups neonatally treated with the noradrenergic neurotoxin, 6-hydroxydopamine (Hård et al., 1982). The treatment caused a slight, statistically non-significant, reduction of the amount of ultra sonic vocalization. Since about 50 per cent of the whole brain levels of NA were spared, the experiment could not conclusively exclude a role for the NA-system in ultra sonic crying.

The main objective of the present study was a renewed investigation on the possible involvement of the NA transmission system in ultra sonic vocalization. The specific rationale for using the α_2-adrenoceptor agonist clonidine in the present study was provided by previous observations indicating an attenuating effect of clonidine on states of distress in both man and animals. This effect is usually attributed to the agonistic effects of clonidine on pre-synaptic α_2-adrenoceptors, inhibiting release of NA in the synaptic cleft.

The main result of the present study indicates that the noradrenergic transmission system is involved in the neural control of ultra sonic vocalization in rat pups. The specific effect of clonidine on crying was however opposite to the one expected. Viewing ultra sonic vocalization as a distress response, previous studies on adult animals suggested an attenuation of this response, whereas we found a reinforcement of the crying. Interestingly, more recent findings also suggest that clonidine at high doses exerts a pro-conflict effect in animal conflict-situations, like Vogel's drinking test and Montgomery's elevated maze (Handley et al., 1984; Söderpalm and Engel, 1988).

In the present study, a series of experiments was performed to analyze the conditions for and the mechanisms mediating the stimulatory effect of clonidine on ultra sonic crying. This series of experiments was started with an ontogenetic study on the effects of clonidine covering the age span from Day 2 to Day 22 after birth. The effect of clonidine on the amount of crying was small and just noticeable up to 4 days of age. At 6 days of age the effect was appreciable and increased with advancing age. Clonidine also substantially increased ultra sonic crying after the age, at about 12 days of age, when the amount of crying normally begins to decrease. Furthermore, even during the age period of disappearance of ultra sonic crying, from about 16 days after birth, clonidine strongly increased the amounts of crying and in fact exerted its strongest effect during that age period. The excitatory effect of clonidine on vocalization was terminated, rather abruptly, at 20 days of age. This event seems to temporally coincide with the establishment of a sedative effect of clonidine, well known in adult animals (Kellogg and Lundborg, 1972; Reinstein and Isaacson, 1977, 1981; Nomura and Segawa, 1979). It is of interest that animals treated with clonidine at this age, showing episodes of cataleptic-like states before or during the test, displayed high amounts of crying immediately after such an episode.

In the case of the sedative action of clonidine on locomotion, established at day 20, both presynaptic (Nomura et al., 1980) and postsynaptic α_2-adrenoceptors (Spyraki and Fibiger, 1982) have been proposed as mediating the clonidine effect.

The present study suggests that the stimulatory effects of clonidine on crying is mediated by postsynaptic adrenoceptors. Thus clonidine stimulated crying in rat pups, devoid of presynaptic catecholamine activity by the combined treatment with reserpine, a monoamine depletor, and α-methyl-tyrosine, an inhibitor of CA-synthesis.

In rat pups, only treated with reserpine and α-methyl-tyrosine, the amount

of crying was strongly reduced compared to untreated pups, similarly exposed to cold stimulation. This finding suggests that ultra sonic vocalization elicited by cold stimulation is mediated by monoaminergic system. Previous studies indicated that one of the monoamines, serotonin, exerted a stimulating action on ultra sonic crying under similar conditions of cold stimulation (Hård et al., 1982). The present results showing a stimulatory action of clonidine on crying in monoamine-depleted pups, suggest that also noradrenaline may be involved in the effect of cold stimulation on ultra sonic vocalization. This is in line with previous studies indicating activation of the NA-system by cold stress stimulation.

In further studies we tried to establish if the post-synaptic adrenoceptors mediating the effect of clonidine and of cold stimulation on crying were of α_1- or of α_2-type. For this purpose we tried to antagonize the effects of clonidine and of cold stimulation by the use of antagonists with selective action either on the α_1-receptors, prazosin, or on the α_2-receptors, idazoxan.

Investigating first the effects of the antagonists on cold-induced crying, it was found that idazoxan, but not prazosin, antagonized this kind of stimulation. These results thus suggest that the ultra sonic callings emitted by rat pups exposed to a cold environment may be mediated by the tonic activation of post-synaptic α_2-adrenoceptors.

In the case of clonidine-induced vocalization the results were, however, somewhat different. At 12 days of age idazoxan antagonized the effect of clonidine on crying only at a dose, which by itself had a strong inhibitory effect on cold-induced vocalization. By contrast, prazosin antagonized the effect of clonidine at a dose not affecting crying induced by cold stimulation alone. These results suggest that the effect of clonidine on ultra sonic vocalization may be partly medited by α_2-adrenoceptors, but also by a group of receptors sensitive to prazosin, presumably α_1-adrenoceptors. These receptors are probably not stimulated by cold stimulation, since the effect of such stimulation was not antagonized by prazosin. Further experiments must clarify the physiological significance of the α_1-receptors for ultra sonic crying and the possible dependence of these receptors on other environmental stimuli affecting ultra sonic vocalization.

At 18 days of age, the antagonistic effect of prazosin on clonidine-induced crying was no longer apparent, whereas the effect of idazoxan persisted and even seemed reinforced. The effect of prazosin was absent also at day 16 indicating that the loss of effect was occurring in the age interval 12 and 16 days of age. The results thus suggest that during this age-interval, the threshold for blocking the effect of clonidine is raising for the α_1-receptors and lowered for the α_2-receptors. Interestingly, this age interval also constitutes a period of transition for the effect of clonidine on locomotor activity. The stimulating effect of clonidine, predominant during early ages, at that age abates to be replaced later on by sedative effects established at about 20 days of age, as mentioned above. These changes in responsiveness to adrenergic agents pre-

sumably reflect maturational changes in the CNS, whose elucidation requires neurochemical investigations, a subject matter attracting increased attention (Morris et al., 1980; Hartley and Seeman, 1983; Nomura et al., 1982, 1984).

In conclusion the present studies indicate that clonidine stimulates ultra sonic vocalization in rat pups during the whole course of preweaning age, except for the immediate neonatal age. The effect of clonidine seems to be mediated by postsynaptic adrenoceptors of both α_1- and α_2-receptors at an earlier age, but subsequently only by α_2-receptors. The ultra sonic vocalization normally elicited by cold stimulation was antagonized by the α_2-antagonist idazoxan, but not by the α_1-adrenoceptor prazosin. This suggests that cold-stimulated crying, vital for the survival of the pup, is mediated by postsynaptically located α_2-adrenoceptors.

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