Pharmacology Biochemistry & Behavior, Vol. 32, pp. 927–932. © Maxwell Pergamon Macmillan plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Possible Role of Alpha-2 and Alpha-1 Adrenoceptors in the Experimentally-Induced Depression of the Central Nervous System

L. G. HARSING, JR.,[1] J. KAPOCSI AND E. S. VIZI

Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest, Hungary

Received 12 July 1988

HARSING, L. G., JR., J. KAPOCSI AND E. S. VIZI. *Possible role of alpha-2 and alpha-1 adrenoceptors in the experimentally-induced depression of the central nervous system.* PHARMACOL BIOCHEM BEHAV **32**(4) 927–932, 1989.—The alpha-2 adrenoceptor agonists clonidine and xylazine were employed in chicks and rats to induce a loss of the righting reflex, a sign for depression of the central nervous system. These effects of clonidine and xylazine were antagonized by yohimbine, idazoxan and CH-38083 (7,8-(methylenedioxi)-14-alpha-hydroxyalloberbane HCl), compounds having alpha-2 adrenoceptor antagonist properties. Prazosin, an antagonist for alpha-1 adrenoceptors, enforced the alpha-2 adrenoceptor agonist-induced depression in both species. 6-Hydroxydopamine treatment, which reduced the norepinephrine concentrations in the rat cerebral cortex by 76%, increased the duration of the loss of righting reflex induced by xylazine indicating that central postsynaptic alpha-2 adrenoceptors might also be involved in this behavioral alteration. The electrically-stimulated tritium release was also determined from the isolated rat cerebral cortex slices which had been preloaded with ^{3}H-norepinephrine. Clonidine and xylazine inhibited the stimulation-induced tritium release and this inhibition was counteracted by yohimbine, idazoxan or CH-38083, but not by prazosin. We have concluded from the present data that stimulation of alpha-2 adrenoceptors with pre- and postsynaptic locations or inhibition of alpha-1 adrenoceptors in the central nervous system may shift the depression/vigilance balance to the direction of depression which might be accompanied by a decreased activity of cortical noradrenergic neural transmission.

Alpha-2 adrenoceptors Alpha-1 adrenoceptors Alpha-2 adrenoceptor agonists Alpha-2 adrenoceptor antagonists Depression CH-38083

SEDATION, sleep and characteristic behavioral signs of depression can be observed in chicks and rodents treated with clonidine or xylazine (3, 8, 11, 12, 19). These effects are apparently related to the agonist action of clonidine and xylazine on central alpha-2 adrenoceptors (12, 19–21). Intraventricular microinjection or microinfusion into the locus coeruleus of yohimbine, an alpha-2 adrenoceptor antagonist, produces behavioral arousal, increase in locomotor and exploratory activity and electrocortical desynchronization (2, 6, 17). Administration of alpha-2 but not alpha-1 adrenoceptor antagonists can counteract the alpha-2 adrenoceptor agonists-induced behavioral changes (3, 4, 12, 19, 20, 23).

Activation of central alpha-2 and alpha-1 adrenoceptors might lead to opposite effects. Thus, selective agonists for alpha-1 adrenoceptors (phenylephrine, methoxamine, cirazoline) have no sedative effect in chicks (20), they produce cardiovascular stimulation of central origine (13), whereas an opposite effect can be seen following alpha-2 adrenoceptor stimulation (19). Evidence has been obtained that local application of methoxamine on rat cortical neurons enhances the neural firing (1). Furthermore, stimulation of alpha-1 adrenoceptors by cirazoline induces arousal (20) and mediates behavioral excitation in depression of the central nervous system (18). It was also shown that central alpha-1 adrenoceptors have a controlling role in the mechanism of cataplexy (15).

In this paper we have employed selective antagonists for alpha-2 and alpha-1 adrenoceptors to investigate the role of these alpha adrenoceptor subtypes in the clonidine- and xylazine-induced depression of the central nervous system. Since evidence has been obtained that the dorsal and ventral ascending noradrenergic pathways which originate from the locus coeruleus are involved in the effects of clonidine and clonidine-like compounds (6), the behavioral changes induced by alpha-2 and alpha-1 adrenoceptor agonists and antagonists were correlated with the effect of these drugs on ^{3}H-norepinephrine release determined from isolated rat cerebral cortex. In addition, CH-38083 (7,8-(methylenedioxi)-14-alpha-hydroxyalloberbane HCl), recently de-

[1]Requests for reprints should be addressed to Laszlo G. Harsing, Jr., M.D., Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest VIII, Szigony u. 43, Hungary.

scribed highly selective alpha-2 adrenoceptor antagonist (26), was used as a tool in this study to investigate the role of alpha adrenoceptor subtypes in the behavioral and neurochemical changes.

METHOD

Experiment I

Groups of 8–10 two-day-old chicks weighing 35–45 g each were used for sleep experiments. The animals were transported into a quiet and warm room where the experiments were performed. Sleep-like behavior was induced by clonidine which was dissolved in saline and injected subcutaneously under the wing in a dose of 0.7 mg/kg. The duration of sleep-like behavior was determined by measuring the time interval between loss of righting reflex and the spontaneous righting of the animals (12). In antagonist experiments yohimbine (0.25–1.0 mg/kg), idazoxan (0.5–4.0 mg/kg), CH-38083 (0.05–0.5 mg/kg) and prazosin (10 mg/kg) were injected intraperitoneally 10 min prior to clonidine administration.

Experiment II

Male Wistar rats weighing 100–120 g were injected with xylazine intravenously in a dose of 2.0 mg/kg. The depression of the central nervous system evoked by xylazine was measured from the loss of the righting reflex till returning of the spontaneous locomotion. The potential antagonists were injected subcutaneously 30 min prior to xylazine administration.

Vehicle or 6-hydroxydopamine (6-OHDA) were injected intracerebroventricularly to rats anesthetized by pentobarbital sodium (30 mg/kg intraperitoneally) and placed into a stereotaxic instrument. Two hundred fifty μg of 6-OHDA dissolved in 20 μl of saline containing 5.6 mmol/l ascorbic acid was infused into the right lateral ventricle through a stainless steel needle placed 2 mm lateral and 1 mm posterior to bregma and 3 mm ventral to the dura. Rats were used for experiments 5 days after treatment with 6-OHDA. Norepinephrine depletion in the rat cerebral cortex evoked by 6-OHDA injection was measured by high performance liquid chromatography/electrochemistry as described by Mefford (14) and Harsing and Vizi (10).

Experiment III

Wistar rats were decapitated and the brain was quickly removed from the skull and slices of the frontal cortex were prepared according to Glowinski and Iversen (9). The tissue was loaded with 1-(7,8-^{3}H)-norepinephrine (370 kBq/ml) for 60 min at 36°C in oxygenized Krebs solution (NaCl 113, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11.5 mmol/l) containing 3×10^{-4} mol/l ascorbic acid and 3×10^{-5} mol/l disodium EDTA. After loading the tissue, cortex slices were transferred into superfusion chambers and superfused at a rate of 1 ml/min for 60 min at 36°C with oxygenized Krebs solution containing ascorbic acid (3×10^{-4} mol/l), disodium EDTA (3×10^{-5} mol/l), cocaine HCl (8×10^{-6} mol/l) and prednisone (3×10^{-6} mol/l). The effluent was discarded in this period then subsequently 3-min samples were collected. Tissues were stimulated electrically (2 Hz frequency, 20 V, 2 msec impulse duration, 360 shocks for 3 min) by the means of an Eltron Electrostimulator (Hungary) in the 4th and 14th collection periods. Alpha adrenoceptor agonists or antagonists studied in this series of experiments were added to the perfusion fluid from the 8th collection period. At the end of the experiments tissues were collected and homogenized in 1 ml ice-cold 0.2 mol/l perchloric acid containing 0.11 mmol/l ascorbic acid and centrifuged at $1500 \times g$ for 10 min. The total radioactivity of the tritiated compounds (^{3}H-norepinephrine and ^{3}H-labelled metabolites) in the perfusate and the tissue was determined by an LKB 1211 Rackbeta Liquid Scintillation Counter.

Statistical Analyses

The duration of sleep-like behavior in antagonism studies was expressed as percentage of the control group receiving clonidine (Experiment I) or xylazine (Experiment II). Differences between the mean values of the control and pretreated groups were evaluated using the Student two-tailed *t*-statistic (12). One-way analysis of variance (ANOVA) and the Dunn test was used for statistical analysis of the ^{3}H-norepinephrine release data (Experiment III). The mean ± S.E.M. was calculated. A level of probability less than 0.05 was considered significant.

Drugs

Xylazine HCl (Bayer, Leverkusen FRG), clonidine HCl (Boehringer Ingelheim, Ingelheim, FRG), yohimbine HCl, 6-hydroxydopamine HCl (Sigma Chemcial Co., St. Louis, MO), prazosin HCl (Pfizer Inc., New York, NY), idazoxane (Reckitt and Colman, Dansom Lane, England), cocaine HCL (Alkaloida, Hungary), prednisone (Organon, Holland). CH-38083 (7,8-(methylenedioxi)-14-alpha- hydroxyalloberbane HCl) was synthetized by Drs. Cs. Szantay and I. Toth, Central Chemical Research Institute, Hungarian Academy of Sciences, Budapest (26,27). 7,8-^{3}H-noradrenaline HCl (specific activity 10.5 Ci/mmol) was purchased from Radiochemical Centre Amersham, England.

RESULTS

Experiment I. Clonidine-Induced Depression in Chicks

Subcutaneous injection of clonidine (0.7 mg/kg) induced loss of the righting reflex in chicks. Characteristic signs of depression were also observed: the animals closed their eyes, the head was bowed and the wings were drooped in response to clonidine. These behavioral changes of the chicks appeared usually within 5–7 min after clonidine administration. The duration of the sleep-like behavior evoked by clonidine was 24.3 ± 3.6, 21.8 ± 2.4 and 26.0 ± 2.1 min in different control groups (mean ± S.E.M., n = 8–10).

Results obtained from antagonism studies are shown in Fig. 1. The alpha-2 adrenoceptor antagonists yohimbine, idazoxan and CH-38083 reduced the duration of clonidine-induced loss of the righting reflex in chicks and this effect proved to be dose-dependent. When the alpha-2 adrenoceptor antagonists were administered by themselves, no behavioral alteration or sleep-like behavior was observed.

The alpha-1 adrenoceptor antagonist prazosin was administered in a dose of 10 mg/kg intraperitoneally 15 min prior to clonidine. Prazosin pretreatment increased the clonidine-induced sleeping time from 32.6 ± 2.5 to 57.2 ± 5.1 min (mean ± S.E.M., n = 10–10). This difference was significant (Student two-tailed *t*-statistic, $p<0.001$). Prazosin failed to induce behavioral changes in chicks which did not receive clonidine.

Experiment II. Xylazine-Induced Depression in Rats

Intravenous injection of xylazine induced depression in rats characterized by immobilization, closed eyes, bowed head and appearance of sleep-like behavior. The animals which received xylazine treatment were completely resistant to external stimulus. The loss of the righting reflex was observed within 1–3 min after xylazine administration. The depressant effect of xylazine was

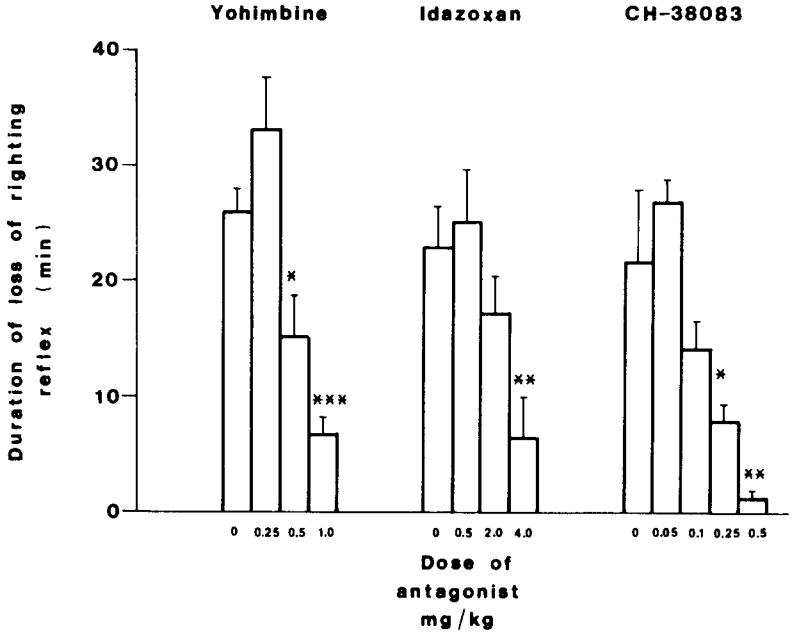

FIG. 1. Effect of alpha-2 adrenoceptor antagonist yohimbine, idazoxan and CH-38083 on clonidine-induced loss of the righting reflex in chicks. Clonidine was injected subcutaneously in a dose of 0.7 mg/kg, alpha-2 adrenoceptor antagonists were given intraperitoneally 10 min prior to clonidine administration. Mean ± S.E.M. for 8–10 chicks, Student two-tailed *t*-statistic, $*p<0.05$, $**p<0.01$, $***p<0.001$ versus clonidine-treated groups.

found to be dose-dependent (Fig. 2). For the antagonism studies the effect of 2 mg/kg of xylazine (IV injection) was chosen which induced a sleep-like behavior lasted 29.1 ± 0.9, 27.0 ± 1.3, 30.6 ± 1.5 and 31.1 ± 1.6 min, in different groups of animals, respectively (mean ± S.E.M., n = 10–10).

As it is shown in Fig. 3, subcutaneous administration of idazoxan and CH-38083 reduced the duration of the loss of the righting reflex in rats evoked by intravenous injection of 2 mg/kg of xylazine. CH-38083 was found to be more potent than idazoxan

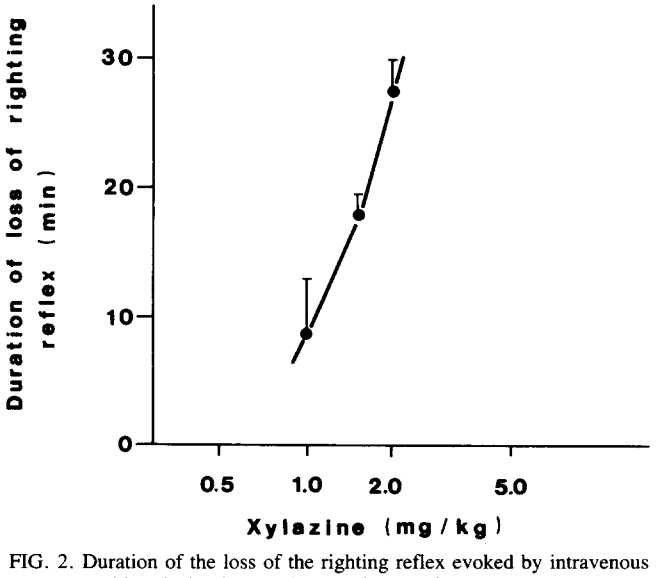

FIG. 2. Duration of the loss of the righting reflex evoked by intravenous treatment with xylazine in rats. Mean ± S.E.M. for 5 to 10 rats.

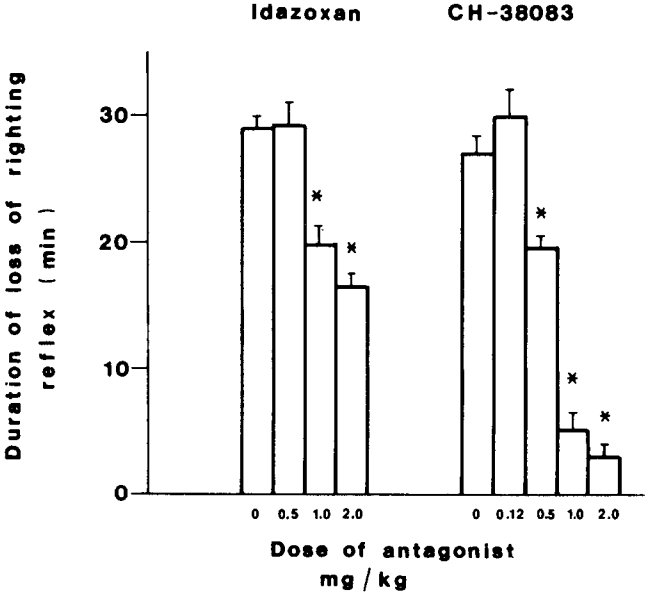

FIG. 3. Effect of alpha-2 adrenoceptor antagonist idazoxan and CH-38083 on xylazine-induced loss of the righting reflex in rats. Xylazine was injected intravenously in a dose of 2.0 mg/kg, alpha-2 adrenoceptor antagonists were given subcutaneously 30 min before xylazine administration. Mean ± S.E.M., for 10 rats, Student two-tailed *t*-statistic, $*p<0.001$ versus xylazine-treated groups.

in antagonizing the depressant effect of xylazine similarly to that observed in chicks. Neither idazoxan nor CH-38083 applied in a dose of 4 mg/kg subcutaneously induced alteration in the behavior of the rat.

Two hundred fifty μg of 6-OHDA injected intracerebroventricularly to rats reduced the norepinephrine concentrations in the frontal cortex from 11.69 ± 0.33 to 0.40 ± 0.07 nmol/g (mean ± S.E.M., n = 5–5, Student two-tailed *t*-statistic, $p<0.01$). Xylazine injected intravenously in a dose of 2 mg/kg induced a longer lasting loss of righting reflex in 6-OHDA-treated rats than in sham-operated rats (40.2 ± 1.2 versus 22.6 ± 1.6 min, mean ± S.E.M., n = 8–9, Student two-tailed *t*-statistic, $p<0.001$). Xylazine induced no behavioral excitation in 6-OHDA-pretreated rats.

The sleep-like behavior evoked by intravenous injection of 2 mg/kg of xylazine was significantly extended by subcutaneous treatment with 5 mg/kg of prazosin: control group, 26.7 ± 1.0 min, prazosin-pretreated group, 43.5 ± 2.3 min (mean ± S.E.M., n = 10–10, Student two-tailed *t*-statistic, $p<0.001$). In other series of experiments rats were treated with 10 mg/kg prazosin subcutaneously. The xylazine- (2 mg/kg IV) induced loss of the righting reflex was increased from 24.8 ± 1.4 to 52.2 ± 1.9 min in this experiment (mean ± S.E.M., n = 10–10, Student two-tailed *t*-statistic, $p<0.001$). Rats treated with prazosin closed their eyes, became immobilized and the sedation developed could be transiently interrupted by touching the animals.

Experiment III. Effect of Alpha Adrenoceptor Agonists and Antagonists on ^{3}H-Norepinephrine Release From Isolated Rat Cerebral Cortex Slices

The spontaneous release of radioactivity measured from isolated rat cerebral cortex slices which had been preloaded with ^{3}H-norepinephrine became stable after a 120-min perfusion. Repeated electrical stimulation resulted in an identical rate of radioactivity efflux from the tissue being the S2/S1 ratio 0.97 ± 0.03 (n = 6) in control conditions. Norepinephrine (5×10^{-7} mol/l) and the alpha-2 adrenoceptor agonists clonidine and xylazine applied in a concentration of 5×10^{-7} mol/l reduced the electrically-stimulated tritium release: the S2/S1 ratios were 0.58 ± 0.10 (n = 6), 0.30 ± 0.03 (n = 4) and 0.64 ± 0.04 (n = 6) for norepinephrine, clonidine and xylazine, respectively, $F(3,18)=14.72$, $p<0.001$ versus control group.

The effects of alpha adrenoceptor antagonists on the tritium release measured from ^{3}H-norepinephrine preloaded rat cerebral cortex slices are summarized in Table 1. Yohimbine (5×10^{-6} mol/l), idazoxan and CH-38083 (5×10^{-7} mol/l) significantly enhanced the electrically-stimulated tritium release from cortical slices of the rat without affecting the resting tritium efflux. In contrast, the alpha-1 adrenoceptor antagonist prazosin, added in a concentration of 10^{-6} mol/l, failed to modify the electrical stimulation-induced tritium release.

The electrically-stimulated tritium release inhibited by 5×10^{-7} mol/l concentration of norepinephrine was counteracted by yohimbine, idazoxan and CH-38083 (Table 1). In these experimental conditions yohimbine was found about 10-fold less potent in respect to alpha adrenoceptor antagonism than idazoxan or CH-38083. The alpha-1 adrenoceptor antagonist prazosin, which did not affect the electrically-induced tritium release from rat cerebral cortex by itself, failed to antagonize the inhibitory effect of norepinephrine on tritium release from cortical slices (Table 1).

TABLE 1

EFFECT OF ALPHA ADRENOCEPTOR ANTAGONISTS ON ELECTRICAL STIMULATION-INDUCED ^{3}H-NOREPINEPHRINE RELEASE (S2/S1) AND THEIR ANTAGONIST EFFECTS ON NOREPINEPHRINE INDUCED INHIBITION FROM ISOLATED RAT CEREBRAL CORTEX SLICES

Compounds	Concentration mol/l	S2/S1
1. None	–	0.93 ± 0.10(7)
2. Yohimbine	5×10^{-6}	1.95 ± 0.38(4)‡
3. Idazoxan	5×10^{-7}	1.54 ± 0.09(4)*
4. CH-38083	5×10^{-7}	1.91 ± 0.23(4)‡
5. Prazosin	10^{-6}	0.84 ± 0.06(4)
1. Norepinephrine	5×10^{-7}	0.66 ± 0.05(4)
2. Norepinephrine	5×10^{-7}	
+ yohimbine	5×10^{-6}	1.36 ± 0.10(4)‡
3. Norepinephrine	5×10^{-7}	
+ idazoxan	5×10^{-7}	1.44 ± 0.11(4)‡
4. Norepinephrine	5×10^{-7}	
+CH-38083	5×10^{-7}	1.20 ± 0.14(4)†
5. Norepinephrine	5×10^{-7}	
+ prazosin	10^{-6}	0.64 ± 0.06(4)

For experimental conditions see Experiment III in the Method section. One-way analysis of variance (ANOVA) and Dunn test, $F(4,18)=8.19$ and $F(4,15)=11.19$ for the 1st and the 2nd series of experiments, respectively, *$p<0.05$, †$p<0.01$, ‡$p<0.001$ versus none treated or norepinephrine-treated groups. Means ± S.E.M., number of experiments in parentheses.

DISCUSSION

Data presented in this paper confirm the earlier observations that stimulation of central alpha-2 adrenoceptors produces sedation defined as loss of the righting reflex and evokes sleep-like behavior in experimental conditions (12, 19, 20). Of the alpha-2 adrenoceptor agonists we applied clonidine and xylazine to induce depression of the central nervous system in young chicks and rats. A rank order of alpha adrenoceptor agonists to cause sedation in rat was reported by Drew *et al.* (8): clonidine > xylazine = naphazoline > methoxamine > phenylephrine. Roach and his co-workers (20) also investigated a great number of alpha-2 adrenoceptor agonists to induce sleep-like state in young chicks and the relative rank order of potencies constructed was UK 14,304 = guanabenz = guanoxabenz > guanfacine > xylazine.

The role of alpha-2 adrenoceptors in the mediation of clonidine- or xylazine-induced depression was substantiated by the fact that yohimbine, piperoxan and idazoxan (7), compounds known to block alpha-2 adrenoceptors, abolished the loss of the righting reflex evoked by these compounds (8, 12, 20). We used yohimbine, idazoxan and CH-38083 for reversal of clonidine- or xylazine-induced loss of the righting reflex in young chicks or rats. Recently, CH-38083, an alloberbane derivative, has been found to be highly selective antagonist for alpha-2 adrenoceptors on isolated rat vas deferens or guinea pig ileum (26,27). Of the alpha-2 adrenoceptor blockers employed in this study, CH-38083 antagonized most effectively the clonidine- or xylazine-induced central nervous system depression evoked in young chicks or rats. This finding supports the earlier suggestion that selective alpha-2 adrenoceptor antagonists can be used to control the sedative state induced by clonidine or xylazine (12).

Using transcortical dialysis it was found that idazoxan enhances cortical norepinephrine release in the rat (5). We obtained similar results by employment of isolated rat cortical slice preparation preloaded with ^{3}H-norepinephrine: yohimbine, idazoxan and CH-38083 increased the electrically-stimulated tritium release. On the contrary, clonidine and xylazine inhibited the tritium release elicited by electrical field stimulation from cortical slices in vitro and induced depression of the central nervous system in

young chicks and rats in vivo. The opposite effect of alpha-2 adrenoceptor agonists and antagonists on ^{3}H-norepinephrine release measured from cortex slices can sufficiently well be explained by stimulation or inhibition of presynaptic alpha-2 adrenoceptors of the noradrenergic axon varicosities which are operative in the feedback inhibition process of the neurotransmitter release. In other series of experiments the alpha-2 adrenoceptor antagonists reversed the exogenous norepinephrine inhibition on cortical ^{3}H-norepinephrine release and in vivo conditions they suspended the central nervous system depression elicited by alpha-2 adrenoceptor stimulation. Thus, one can speculate from the present data that a possible correlation exists between the presynaptic alpha-2 adrenoceptor-mediated noradrenergic neural activity in the central nervous system and the actual state of depression and vigilance.

Convincing evidence is available that there is a functional interaction between noradrenergic ascending systems and other neurons also equipped with alpha-2 adrenoceptors (25). This kind of alpha-2 adrenoceptors are located postsynaptically in relation to noradrenergic axon terminals and they might have a regulatory role in mediation of other neurotransmitters released at cortical levels. Stimulation of locus coeruleus, for example, results in an increase of GABA release (16) and a decrease of acetylcholine release (24) from the cerebral cortex. The importance of the postsynaptic alpha-2 adrenoceptors in behavioral alterations is shown by the fact that 6-OHDA pretreatment increased the xylazine-induced depression of the central nervous system in the rat. The increase in length of depression evoked by xylazine is probably due to development of 6-OHDA treatment-induced denervation supersensitivity of the postsynaptic alpha-2 adrenoceptors. It is worthwhile to note that clonidine induced locomotor hyperactivity in 6-OHDA + reserpine-pretreated rats, but no such behavior was found after clonidine given to animals pretreated with 6-OHDA alone (28). Therefore, it is plausible to suggest that alpha-2 adrenoceptor agonists, when they evoke depression, stimulate both pre- and postsynaptic alpha-2 adrenoceptors, which leads to inhibition of norepinephrine release, and on the other hand, to stimulation or inhibition of other neurons in the network through their own alpha-2 adrenoceptors. We speculated that alpha-2 adrenoceptor antagonists exert opposite actions: they block both pre- and postsynaptic alpha-2 adrenoceptors by which they increase the release of norepinephrine and suspend the postsynaptic alpha-2 adrenoceptors-mediated responses (i.e., sedation). The enhanced release of norepinephrine induced by alpha-2 adrenoceptor antagonists leads to stimulation of excitatory alpha-1 adrenoceptors (17) and this indirect action might also participate in the reversal of alpha-2 agonists-induced depression.

Evidence was also obtained for the role of alpha-1 adrenoceptors in the state of vigilance and sleep (22). It was reported that prazosin, an antagonist for alpha-1 adrenoceptors, did not reverse the xylazine-induced sleep-like behavior in young chicks, even potentiated its sedative effect (12,20). In our experimental conditions, prazosin also enhanced the duration of the loss of the righting reflex induced by clonidine in chicks or by xylazine in rats. This action of prazosin is probably postsynaptic in nature since this compound did not affect the electrical stimulation induced ^{3}H-norepinephrine release in cortical slice preparation and also failed to reverse the norepinephrine inhibition of ^{3}H-norepinephrine release. Pichler and Kobinger (17) concluded that in mice excitatory alpha adrenoceptors are of alpha-1 type and Clineschmidt and his co-workers (2) reported an increased motor activity in rats elicited by intracisternally administered methoxamine. The excitatory effect of alpha-1 adrenoceptor stimulation at the level of cerebral cortex is also suggested by microiontophoretic studies in which the local application of methoxamine on rat cortical neurons was shown to produce an increase in neuronal firing (1)

In conclusion, noradrenergic pathway arising from the locus coeruleus plays a critical role in behavioral plasticity and its effect is mediated via both alpha-2 and alpha-1 adrenoceptors. If norepinephrine is once released from the axon varicosities it may have an effect on target cells: 1) through alpha-1 adrenoceptor stimulation it might produce excitation and may be responsible for the change of depression/vigilance balance into the direction of vigilance, 2) through alpha-2 adrenoceptors norepinephrine might exert a tonic inhibitory control on its own release and affect indirectly the release of other neurotransmitters from neurons possessing postsynaptic alpha-2 adrenoceptors. The latter type of central alpha-2 adrenoceptors might have significance in mediation of sedative state.

REFERENCES

1. Bradshaw, C. M.; Pun, R. Y. K.; Stater, N. T.; Szabadi, E. Comparison of the effect of methoxamine with those of noradrenaline and phenylephrine on single cerebral cortical neurones. Br. J. Pharmacol. 73:47–54; 1981.
2. Clineschmidt, B. V.; Flataker, L. M.; Faison, E. P.; Haubrich, D. R. Alpha-methyldopa reduces locomotor activity in rats via its metabolite, alpha-methylnoradrenaline, acting on alpha-2 adrenoceptors. Arch. Int. Pharmacodyn. 244:231–243; 1980.
3. Delbarre, B.; Schmitt, H. Sedative effects of alpha sympathomimetic drugs and their antagonism by adrenergic and cholinergic blocking drugs. Eur. J. Pharmacol. 13:356–363; 1971.
4. Delbarre, B.; Schmitt, H. A further attempt to characterize sedative receptors activated by clonidine in chicks and mice. Eur. J. Pharmacol. 22:355–359; 1973.
5. Dennis, T.; Heureux, R. L.; Carter, C.; Scatton, B. Presynaptic alpha-2 adrenoceptors play a major role in the effects of idazoxan on cortical noradrenaline release (as measured by *in vivo* dialysis) in the rat. J. Pharmacol. Exp. Ther. 241:642–649; 1987.
6. De Sarro, G. B.; Ascioti, C.; Froio, F.; Libri, V.; Nistico, G. Evidence that locus coeruleus is the site where clonidine and drugs acting at alpha-1 and alpha-2 adrenoceptors affect sleep and arousal mechanisms. Br. J. Pharmacol 90:675–685; 1987.
7. Doxey, J. C.; Roach, A. G.; Smith, C. F. C. Studies on RX 781094 a selective, potent and specific antagonist of alpha-2 adrenoceptors. Br. J. Pharmacol. 78:489–505; 1983.
8. Drew, G. M.; Gower, A. J.; Marriott, A. S. Alpha-2 adrenoceptors mediate clonidine-induced sedation in the rat. Br. J. Pharmacol. 67:133–141; 1979.
9. Glowinski, J.; Iversen, L. L. Regional studies of catecholamines in the rat brain. I. The disposition of ^{3}H-norepinephrine, ^{3}H-dopamine and ^{3}H-dopa in various regions of the brain. J. Neurochem. 13: 655–669; 1966.
10. Harsing, L. G., Jr.; Vizi, E. S. Release of endogenous dopamine from rat isolated striatum: effect of clorgyline and (−)deprenyl. Br. J. Pharmacol. 83:741–749; 1984.
11. Holman, P. B.; Shillito, E. E.; Vogt, M. Sleep produced by clonidine (2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride). Br. J. Pharmacol. 43:685–695; 1971.
12. Hsu, W. H. Xylazine-induced depression and its antagonism by alpha adrenergic blocking agents. J. Pharmacol. Exp. Ther. 218:188–192; 1981.
13. Huchet, A.-M.; Hugnet, F.; Ostermann, G.; Bakri-Logeais, F.; Schmitt, H.; Narcisse, G. Central alpha-1 adrenoceptors and cardiovascular control in normotensive and spontaneously hypertensive rats. Eur. J. Pharmacol. 95:207–213; 1983.
14. Mefford, I. Application of high performance liquid chromatography with electrochemical detection to neurochemical analysis: measurement of catecholamines, serotonin and metabolites in rat brain. J. Neurosci. Methods 3:207–224; 1981.
15. Mignot, E.; Guilleminault, C.; Bowersox, S.; Rappaport, A.; De-

ment, W. C. Effect of alpha-1 adrenoceptors blockade with prazosin in canine narcolepsy. Brain Res. 444:184–188; 1988.
16. Moroni, F.; Bianchi, C.; Moneti, G.; Tanganelli, S.; Spidalieri, G.; Guandalini, P.; Beani, L. Release of GABA from the guinea-pig neocortex induced by electrical stimulation of the locus coeruleus or by norepinephrine. Brain Res. 232:216–221; 1982.
17. Pichler, L.; Kobinger, W. Modulation of motor activity by alpha-1 and alpha-2 adrenoceptor stimulation in mice. Naunyn Schmiedebergs Arch. Pharmacol. 317:180–182; 1981.
18. Pichler, L.; Kobinger, W. Possible function of alpha-1 adrenoceptors in the CNS in anaesthetized and conscious animals. Eur. J. Pharmacol. 107:305–311; 1985.
19. Roach, A. G.; Lefevre-Borg, F.; Gomeniand, R.; Cavero, I. Pharmacological evidence favoring a similarity between alpha adrenoceptors mediating sleep in chicks and cardiac presynaptic alpha-2 receptors in rat. J. Pharmacol. Exp. Ther. 222:680–687; 1982.
20. Roach, A. G.; Doxey, J. C.; Strachan, D. A.; Cavero, I. Sleeping times evoked by alpha adrenoceptor agonists in two-day-old chicks: an experimental model to evaluate full and partial agonists at central alpha-2 adrenoceptors. J. Pharmacol. Exp. Ther. 227:421–428; 1983.
21. Ruskoaho, H.; Karppanen, H. Xylazine induced sedation in chicks is inhibited by opiate receptor antagonists. Eur. J. Pharmacol. 100: 91–96; 1984.
22. Stenberg, D.; Hilakivi, I. Alpha-1 and alpha-2 adrenergic modulation of vigilance and sleep. In: Wauquier, A.; Gaillard, J. M.; Monti, J. M.; Radulovacki, M., eds. Sleep: Neurotransmitters and neuromodulators. New York: Raven Press; 1985.
23. Timmermans, P. B. M. W. M.; Schoop, A. M. C.; Kwa, H. Y.; van Zwieten, P. A. Characterization of alpha-adrenoceptors participating in the central hypotensive and sedative effects of clonidine using yohimbine, rauwolscine and corynanthine. Eur. J. Pharmacol. 70: 7–15; 1981.
24. Vizi, E. S. Modulation of cortical release of acetylcholine by noradrenaline released from nerves arising from the rat locus coeruleus. Neuroscience 5:2139–2144; 1980.
25. Vizi, E. S. Non-synaptic interactions between neurons. Chichester: John Wiley and Sons; 1984.
26. Vizi, E. S.; Harsing, L. G., Jr.; Gaal, J.; Kapocsi, J.; Bernath, S.; Somogyi, G. T. CH-38083, a selective, potent antagonist of alpha-2 adrenoceptors. J. Pharmacol. Exp. Ther. 238:701–706; 1986.
27. Vizi, E. S.; Toth, I.; Somogyi, G. T.; Szabo, L.; Harsing, L. G., Jr.; Szantay, Cs. Berbanes: A new class of selective alpha-2 adrenoceptor antagonists. J. Med. Chem. 30:1355–1359; 1987.
28. Zebrowska-Lupina, I.; Przegalinski, E.; Sloniec, M.; Kleinrock, Z. Clonidine-induced locomotor hyperactivity in rats. The role of central postsynaptic alpha-adrenoceptors. Naunyn Schmiedebergs Arch. Pharmacol. 297:227–231; 1977.