ELSEVIER

Pharmacology Biochemistry and Behavior, Vol. 54, No. 1, pp. 241-248, 1996

0091-3057/96 $15.00 + .00

SSDI 0091-3057(95)02132-9

Expression of Fos Protein in Various Rat Brain Areas Following Acute Nicotine and Diazepam

OUTI SALMINEN, SIRPA LAHTINEN AND LIISA AHTEE[1]

Division of Pharmacology & Toxicology, Department of Pharmacy, P.O. Box 56, (Viikinkaari 5), FIN-00014 University of Helsinki, Finland

SALMINEN, O., S. LAHTINEN AND L. AHTEE. *Expression of Fos protein in various rat brain areas following acute nicotine and diazepam.* PHARMACOL BIOCHEM BEHAV **54**(1) 241-248, 1996. – We studied the effects of an acute dose of (−)-nicotine (1 mg/kg) on Fos-like immunostaining (IS) in rat brain areas. Nicotine increased Fos IS significantly in the medial terminal nucleus of accessory optic tract (MT), and tended to increase it in the interpeduncular nucleus (IP), as well as in the stress-related areas, the paraventricular hypothalamic nucleus (PVN) and the supraoptic nucleus (SON). Previously nicotine was reported to increase Fos IS also in another stress-related area, the central nucleus of amygdala (ACe). This led us to study the interaction of nicotine with diazepam (10 mg/kg). Diazepam alone increased Fos IS in PVN and in SON as well as in ACe. In diazepam- and nicotine-treated rats Fos IS was increased in PVN and SON as well as in MT and IP. In MT and IP of diazepam and nicotine-treated rats Fos IS was similar to that induced by nicotine alone, and in PVN and SON of these rats Fos IS was similar to that induced by diazepam alone. Nicotine tended to antagonise the diazepam-induced elevation of Fos IS in ACe. Taken together, diazepam induced Fos IS in all stress-related areas studied (PVN, SON, ACe), but not in central visual structures, where nicotine induces Fos IS (MT, IP). No significant interactions on Fos expression were found between acute effects of diazepam and nicotine suggesting that these drugs activate different sets of neurons within the stress-related brain areas.

Nicotine Diazepam c-*fos* Immunohistochemistry Stress

FOS is a nuclear transcription factor protein encoded by an immediate early gene c-*fos*, and it is an early marker of neuronal activation. Fos is known to act in heterodimeric complex with the members of the Jun family of nuclear proteins to control the transcriptional activity of other genes (10). The expression of Fos protein can be induced by a variety of physiological and pharmacological stimuli (11,17,28,29). Fos protein is believed to act as an initiator of long term cellular changes (neural plasticity) in response to these stimuli. Fos expression is sufficiently rapid and transient to map the structures of brain and spinal cord activated after different kinds of stimulation (13,29).

Acute administration of nicotine increases Fos-like immunostaining (Fos IS) in various rat brain areas. High levels of Fos IS are seen in the superficial gray layer of superior colliculus (SuG), the medial terminal nucleus of the accessory optic tract (MT), and the interpeduncular nucleus (IP) (23,27). Further, nicotine has been reported to induce the Fos expression in the parvocellular paraventricular nucleus (pcPVN), in the supraoptic nucleus (SON), as well as in the central nucleus of amygdala (ACe) (21). Nicotine may induce c-*fos* expression by both direct and indirect mechanisms. It may stimulate neurons expressing nicotinic binding sites or induce release of noradrenaline with consequent activation of neuronal adrenergic receptors (31). The behavioral responses to nicotine are also suggested to affect c-*fos* expression (31). mRNA for different subunits of nicotinic receptors is localized in the soma of several nuclei of the ventral tegmental areas, including MT and IP (35). Indeed, in a quantitative in vivo [^{3}H]-nicotine binding study (20) the density of nicotine binding sites in IP was one of the highest (27.8 fmol/mg tissue) of any brain region assayed. Nicotine potently and selectively activates brain stem catecholaminergic regions that project to pcPVN and SON, which regulate the hypothalamo-pituitary-adrenal (HPA) axis and the vasopressin/oxytocin secretion, respectively (21). ACe is a limbic system nucleus that also receives catecholaminergic input from the brain stem (22).

Exposure to different stressful stimuli (immobilization

[1] To whom requests for reprints should be addressed.

stress, hypertonic stress, nociceptive activation with capsaicin, and conditioned aversion) increases the Fos expression in PVN, SON, and ACe among several other brain areas (4,7, 8,16,32). The ability to cope with stress in mammals is known to involve the HPA axis; the secretion of corticotrophin (ACTH) from the pituitary and that of glucocorticoids from the adrenal gland are stimulated. The corticotrophin releasing factor (CRF) containing neurons in pcPVN form the main regulatory center for glucocorticoid secretion in the brain (34). Glucocorticoids mediate the changes in energy and metabolism required for coping with stress. ACe is also involved in the secretion of glucocorticoids (14), and in several stress-induced behavioral and autonomic responses (5) that may depend on central catecholamine release. SON contains mostly neurons releasing vasopressin, which markedly potentiates the effect of CRF on ACTH release (26). Cholinergic innervation mediates vasopressin release within SON (1).

Thus, it seems that nicotine is able to increase Fos expression in several brain areas that are likely to be involved in the coping with stress. Therefore, we wanted to clarify further the nature of nicotine-induced Fos expression in the brain by administering diazepam acutely together with nicotine. Diazepam acts as a tranquilizer, and it has been reported to prevent stress-induced c-*fos* expression completely or partially in several brain areas (4,12). Thus, we were interested to see whether it would interact with the effect of nicotine on Fos expression. Such an interaction would suggest that the neural pathways activated by stressful stimuli might be similar to those activated by nicotine. The dose 10 mg/kg of diazepam was selected on the basis of literature (4). Previously, the effect of diazepam alone on Fos IS in the stress-related brain areas, PVN, SON, and ACe, has not been reported.

METHOD

Animals

The subjects were male Wistar rats bred in our institute. Rats aged 10–12 weeks weighing 250–400 g were housed five to a cage and kept on standard diet and tap water ad lib. Lights were on from 0600 to 1800 h.

Drugs

(−)-Nicotine base (Fluka AG, Buchs SG, Switzerland) was diluted with 0.9% NaCl (saline) and the pH of the final solution was adjusted to 7.0–7.4 with 0.05 M HCl prepared in saline. The dose of 1 mg/kg was selected, because nicotine at this dose has been reported to increase significantly the Fos IS in several brain areas (24,27). Hexamethonium hydrobromide (Fluka AG, Buchs SG, Switzerland) was dissolved in saline. Diazepam solution (Diapam® 5 mg/ml inject., Orion Pharmaceutica, Espoo, Finland) and sodium pentobarbital solution (Mebunat® 60 mg/ml inject., Orion Pharmaceutica) were used as such. The nicotine, hexamethonium, and diazepam solutions were administered in a volume of 2 ml/kg and sodium pentobarbital in 1.5 ml/kg. All doses given refer to the base.

Treatments

Rats were divided to four treatment groups: control group, acute nicotine group (NIC; 1 mg/kg SC, 45 min), acute diazepam group (DZ; 10 mg/kg IP, 55 min) and diazepam + nicotine group (DZ + NIC). Time schedule of drug injections is given in Table 1. Hexamethonium (HM; 10 mg/kg IP, 70 min) was used to prevent the peripheral effects of nicotine, and was given to all rats including the control and diazepam-treated ones.

Tissue Preparation

Rats were deeply anesthetized with sodium pentobarbital (100 mg/kg) and perfused intracardially with 0.9% phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in 0.1 M sodium phosphate buffer (PB) pH 7.4. The brains were postfixed with same fixative for 2 h at the room temperature after perfusion. Brains were immersed in 20% sucrose solution overnight at 8°C. Sections (40 μm) were cut on a cryostat.

Immunohistochemistry

Sections were first incubated in 1.5% normal goat serum (in 0.1 M PB) for 60 min to block nonspecific staining. Sections were then incubated in primary *fos*-antibody diluted 1 : 2000 in PBS (pH 7.2; 0.1% sodiumazide) for 14–16 h at room temperature. Sheep polyclonal antibody to *fos* oncoproteins to a synthetic peptide with amino acid sequence: Met-Phe-Ser-Gly-Phe-Asn-Ala-Asp-Tyr-Glu-Ala-Ser-Ser-Ser-Arg-Cys, selected from a conserved region of mouse and human c-*fos* (33), was obtained from Cambridge Research Biochemicals, Cheshire, UK. Sections were processed with the avidin-biotin method (Vectastain Kit, Vector Laboratories, Burlingame, CA) with diaminobenzidine as the chromagen, mounted on gelatine/chrome alume-coated slides, air dried, dehydrated through graded ethanols to xylene, and coverslipped with DePex (BDH Laboratory Supplies, Poole, UK). Controls for the immunostaining, which included omission of primary antibody, demonstrated no Fos IS.

TABLE 1

THE TIME SCHEDULE OF ANIMAL EXPERIMENTS

	Treatments				
Groups	0 min	15 min	25 min	60 min	70 min
Group 1 (control)	HM	NaCl	NaCl	PB	perf
Group 2 (acute NIC)	HM	NaCl	NIC	PB	perf
Group 3 (acute DZ)	HM	DZ	NaCl	PB	perf
Group 4 (NIC + DZ)	HM	DZ	NIC	PB	perf

HM = hexamethonium (10 mg/kg IP); NaCl = saline, IP or SC; DZ = diazepam (10 mg/kg IP); NIC = nicotine (1 mg/kg SC); PB = pentobarbital (100 mg/kg IP); perf = intracardial perfusion.

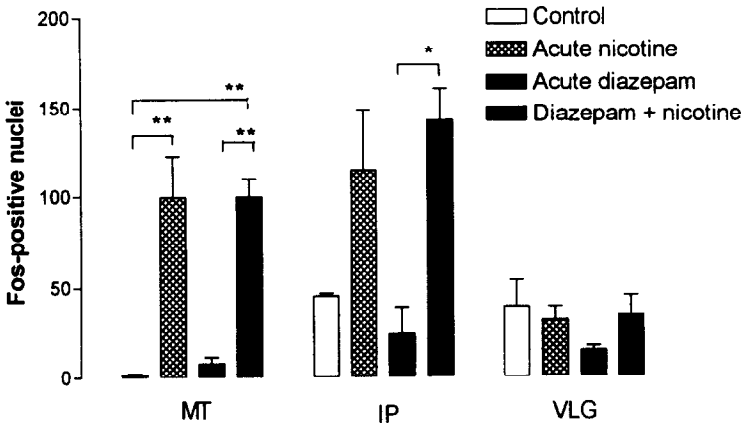

FIG. 1. Expression of Fos protein in areas related to optic system in rats after acute treatment with nicotine (1 mg/kg, SC), diazepam (10 mg/kg, IP) or their combination. The columns give the number of Fos positive nuclei (n = 3-4), the vertical bars show SEM. MT = the medial terminal nucleus of the accessory optic tract; IP = the interpeduncular nucleus; VLG = the ventral lateral geniculate nucleus. *$p < 0.05$; **$p < 0.01$ (Mann-Whitney U-test).

Data Handling and Statistics

The number of Fos-positive nuclei were counted manually using a light microscope at 10 × magnification. A group mean (±SEM) was determined from the counts of three to four rats in each treatment group. The atlas of Paxinos and Watson (24) was used to identify various brain areas. Data were analyzed by Kruskall-Wallis nonparametric ANOVA followed by Mann-Whitney U-test. Results were considered significant at $p < 0.05$.

RESULTS

The Effect of Acute Nicotine on Fos Expression

Areas related to optic system. Nicotine tended to increase Fos IS in IP (number of Fos-positive nuclei 115 ± 34, mean ± SEM, n = 3) as compared with control rats treated with saline under the same conditions (45 ± 2, n = 3). In MT where Fos-positive nuclei were found in only one control animal the increase from 1 ± 1 (n = 4) to 96 ± 23 (n = 4) was statistically significant ($p < 0.01$) (Fig. 1). Fos IS was studied also in two other areas included in the central visual system. However, no Fos IS was detected in SuG of either control or nicotine-treated rats (data not shown), and nicotine did not increase the number of Fos-positive nuclei in the ventral lateral geniculate nucleus (VLG; saline: 39 ± 15, NIC: 32 ± 8, n = 4).

Stress-related areas. Nicotine tended to increase Fos IS in PVN (NIC: 336 ± 54 vs. saline: 281 ± 33, n = 4) (Figs. 2 and 3) and SON (NIC: 89 ± 7 vs. saline: 44 ± 19, n = 4) (Figs. 2 and 4), which hypothalamic areas are related to stress. Nicotine did not induce Fos IS in ACe (100 ± 13, n = 4) as compared with control rats (95 ± 21, n = 4) (Figs. 2 and 5).

The Effect of Acute Diazepam on Fos Expression

Areas related to optic system. In IP (DZ: 24 ± 15 vs. saline: 45 ± 2, n = 3) as well as in VLG (DZ: 15 ± 3 vs. saline 39 ± 15, n = 4) diazepam alone tended to decrease Fos expression. Diazepam did not alter the Fos IS in MT (DZ: 8 ± 4 vs. saline: 1 ± 1, n = 4) (Fig. 1).

Stress-related areas. As compared with control sections diazepam alone increased Fos IS in PVN (DZ: 364 ± 28 vs. saline: 218 ± 33, n = 4, $p < 0.05$) (Fig. 3) and in SON (DZ: 114 ± 10 vs. saline: 44 ± 19, n = 4, $p < 0.05$) (Fig. 4) as well as in ACe (DZ: 167 ± 28 vs. saline 95 ± 21, n = 4, $p < 0.05$) (Figs. 2 and 5).

The Interaction of Nicotine and Diazepam on Fos Expression

Areas related to optic system. In MT of rats treated with diazepam and nicotine the Fos IS was elevated significantly (100 ± 10, n = 4, $p < 0.01$) as compared with control rats (1 ± 1, n = 4), and the number of Fos-positive nuclei was

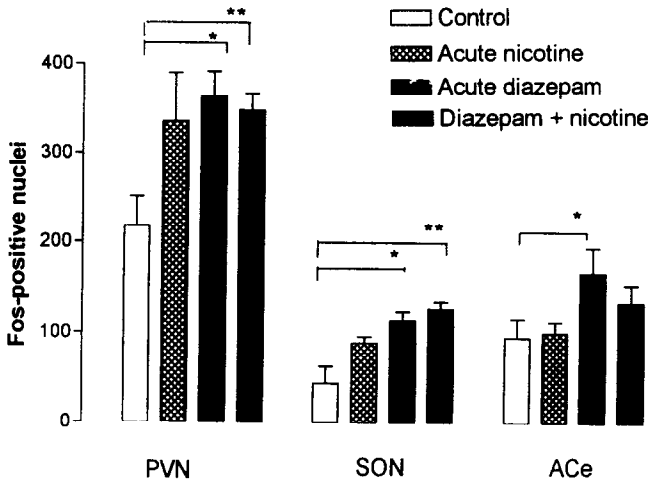

FIG. 2. Expression of Fos protein in areas related to stress in rats after acute treatment with nicotine (1 mg/kg, SC), diazepam (10 mg/kg, IP) or their combination. The columns give the number of Fos-positive nuclei (n = 3-4), the vertical bars show SEM. PVN = the hypothalamic paraventricular nucleus; SON = supraoptic nucleus and ACe = the central nucleus of amygdala. *$p < 0.05$; **$p < 0.01$ (Mann-Whitney U-test).

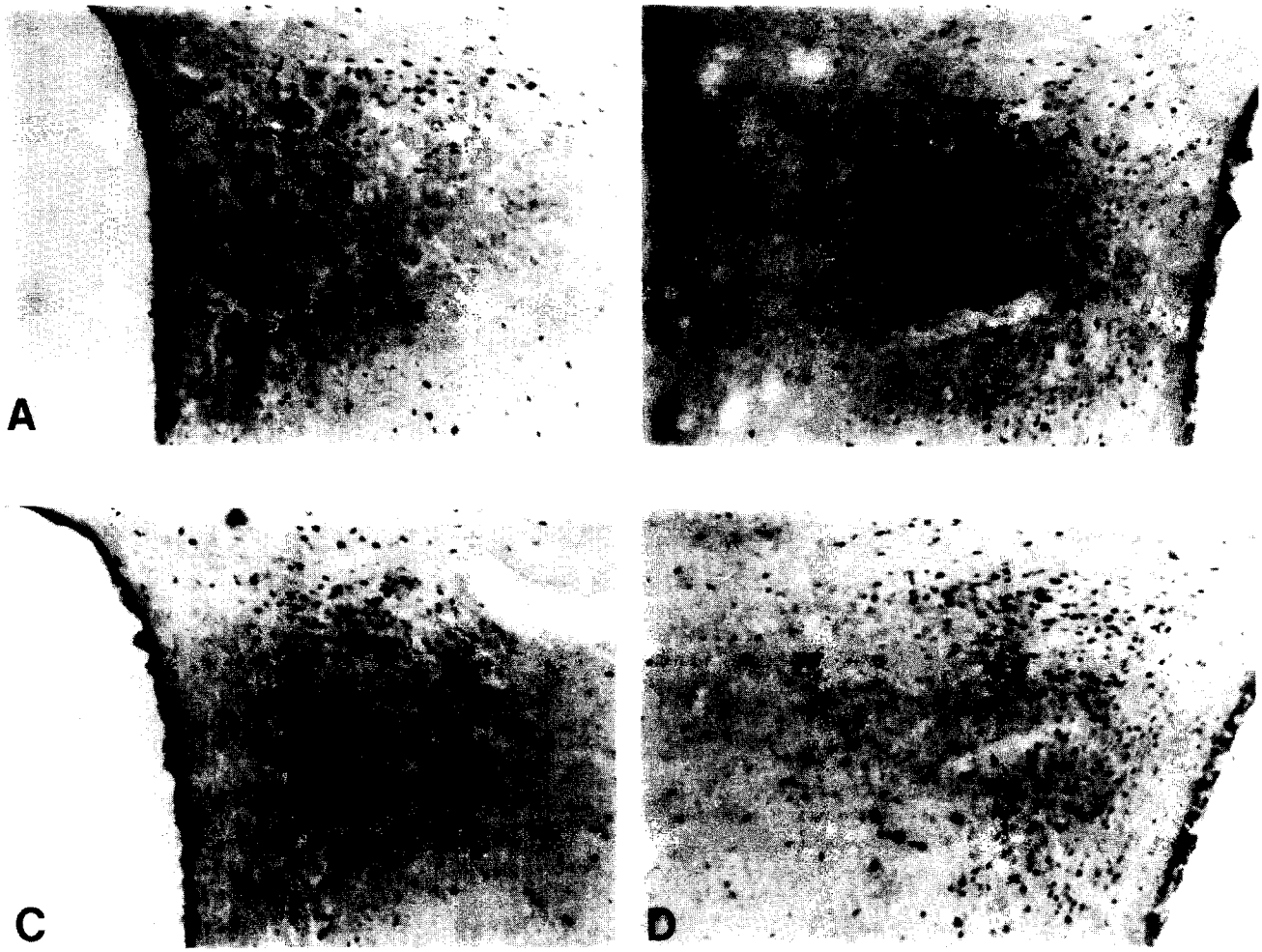

FIG. 3. Immunohistochemical localization of Fos expression in the paraventricular hypothalamic nucleus (PVN). (A) Control. (B) Acute nicotine. (C) Acute diazepam. (D) Diazepam + nicotine. For details of the treatments, see Table 1.

similar to that in rats treated with nicotine (96 ± 23, $n = 4$). In IP of rats given both diazepam and nicotine the Fos IS was elevated (143 ± 18 vs. saline: 45 ± 2; $n = 3$, $p < 0.05$ as compared with DZ group) to about a similar degree as by nicotine alone (Fig. 1).

Stress-related areas. In rats treated with both diazepam and nicotine the Fos IS was significantly increased in PVN (347 ± 19; $n = 4$, $p < 0.01$) as compared with saline-treated control rats (218 ± 33, $n = 4$), but did not differ from those of diazepam (363 ± 28) or nicotine (336 ± 54) alone (Fig. 3). Similarly, in SON of rats given both nicotine and diazepam the Fos IS was significantly elevated as compared with saline rats (DZ + NIC: 127 ± 8 vs. saline: 44 ± 19, $n = 4$, $p = 0.01$) (Fig. 4). Nicotine in combination with diazepam elevated Fos IS in ACe to about same degree as diazepam alone (DZ: 167 ± 30; DZ + NIC 134 ± 22, $n = 4$) (Figs. 2 and 5).

DISCUSSION

Similarly to Ren and Sagar (27) we found that nicotine induces Fos expression in MT and IP, which brain areas are related to visual system. However, in contrast to these authors our study failed to detect Fos IS in SuG. Further, as found by Matta et al. (21) we found that nicotine increases Fos expression in SON and PVN, which hypothalamic areas are related to stress as discussed in the Introduction. Further, Matta et al. (21) also found that IV nicotine activated another stress-related area, ACe. Our study differs from that of Matta et al. (21) in that we pretreated the rats with hexamethonium to prevent the peripheral effects of nicotine. It is possible that this pretreatment masked the effect of nicotine, because hexamethonium alone increased the number of Fos-positive nuclei in the ACe of the control rats (data not given). The nonexistence of the Fos IS in the SuG in our study is most probably due to the different sensitivity of the antibody we used from the one in the study of Ren and Sagar. It is to be noted that the antibodies employed to detect the presence of Fos-protein as well as the incubation times used vary from study to study.

High levels of nicotine binding sites have been reported both in MT and IP (9,35). The nicotine receptors in the MT are predominantly presynaptic (19). Nicotine most probably acts presynaptically or in the retina itself to stimulate the re-

lease of unknown neurotransmitter from retinal ganglia cell axon terminals in MT (27). IP receives a dual cholinergic innervation from the medial habenula and from neurons of the diagonal Broca area (30). In the IP there exist both presynaptic and postsynaptic nicotinic receptors that were found to be heterogenous in regard to subtype (35,36). Also, the medial habenula is rich in nicotinic binding sites (18). Therefore, the site at which nicotine acts to induce Fos expression in IP is unknown (27). Nicotine stimulates the HPA axis resulting in the rapid secretion of ACTH (2). This secretion is mediated centrally via brain stem activation of catecholaminergic afferents to the pcPVN (21). Nicotine is also well known to elevate systemic vasopressin levels (2,37). In SON, nicotine activates dose-dependently both vasopressinergic and oxytocinergic neurons as determined by the Fos expression (21). Nicotine may activate ACe directly or by first stimulating the catecholaminergic inputs to ACe in the brain stem (21). Thus, the effects of nicotine and stress have much in common.

The findings that nicotine increases Fos expression in stress-related brain areas PVN, SON, and ACe led us to study the effects of diazepam on the nicotine-induced Fos expression. In previous studies diazepam pretreatment prevented completely or partially the stress-induced c-*fos* expression in several brain structures including thalamic and hypothalamic nuclei (4) as well as the restraint stress-induced c-*fos* expression in ventral tegmental area (12).

First we studied the effects of diazepam alone at a dose (10 mg/kg) that is reported to suppress the stress-induced Fos expression (4). We found that diazepam alone activated Fos IS in these stress-related areas. However, it did not increase Fos IS in the areas MT, IP, and VLG. In fact, diazepam tended to decrease Fos IS in IP, which is connected to the optic system (36) as well as in VLG, which like MT is part of the accessory optic fiber system in the rat (15). Thus, in this study we report for the first time that acute administration of diazepam alone increased the number of Fos-positive nuclei significantly in PVN, SON, and ACe. In these areas different kinds of stress (restraint stress or capsaicin injection, which leads to general stress response) also induce high levels of Fos immunostaining (3,16). One limitation of this applied method is that the expression of Fos protein cannot be used to determine the function of labeled cells (6). However, our Fos expression studies indicate that diazepam activates neuronal pathways in stress-related areas. Indeed, diazepam at the dose

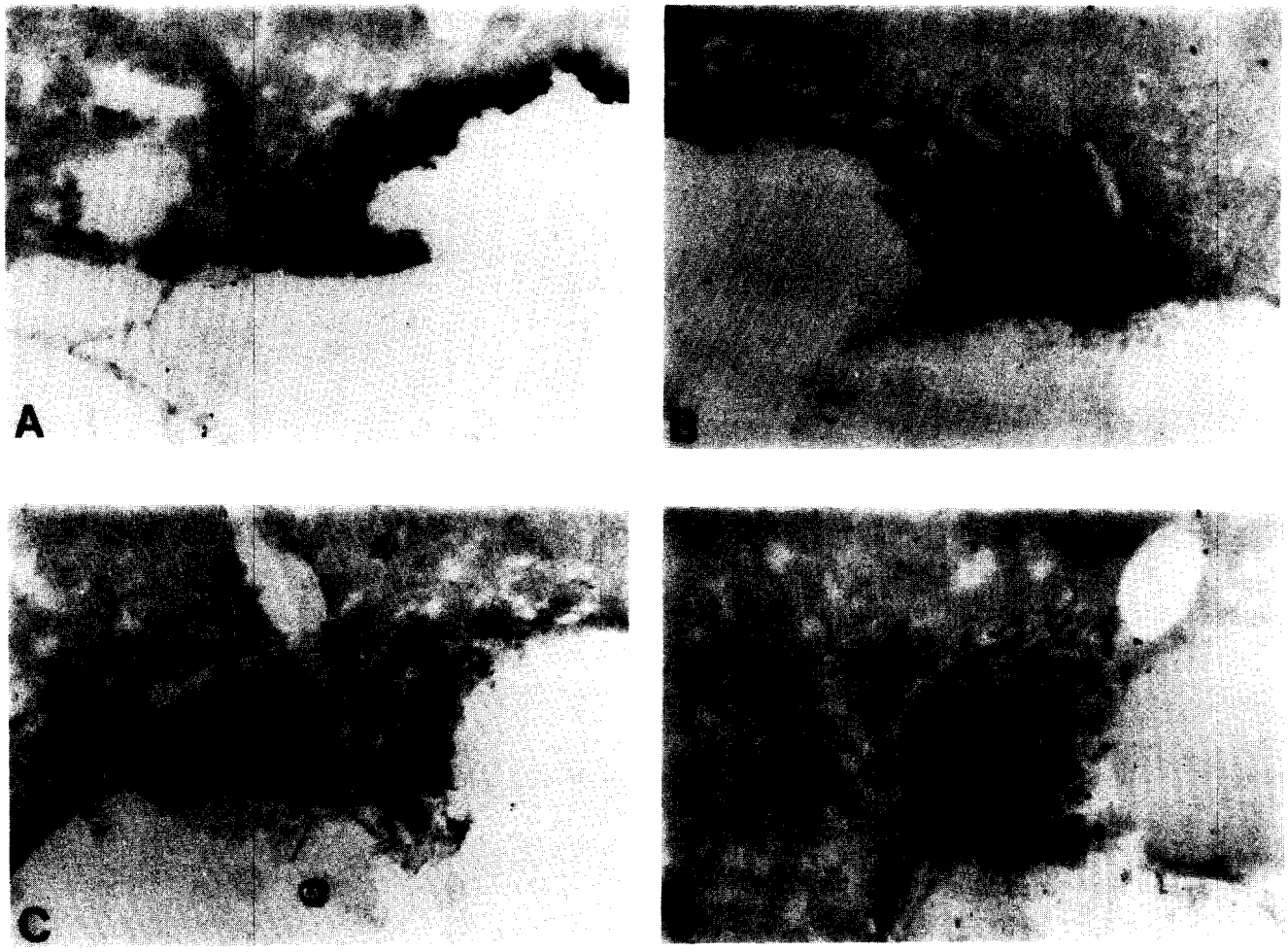

FIG. 4. Immunohistochemical localization of Fos expression in the supraoptic nucleus (SON). (A) Control. (B) Acute nicotine. (C) Acute diazepam. (D) Diazepam + nicotine. For details of the treatments, see Table 1.

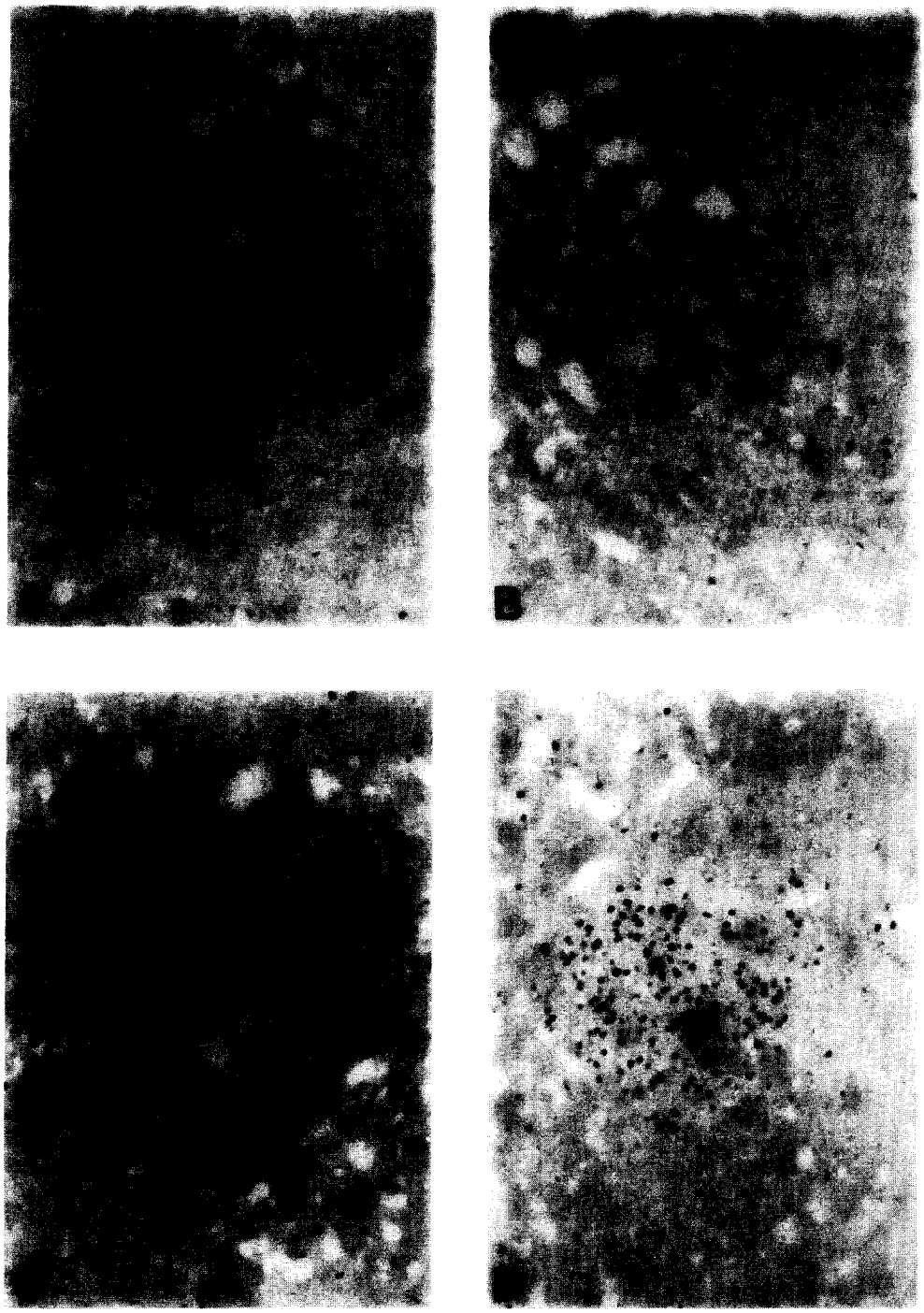

FIG. 5. Immunohistochemical localization of Fos expression in the central nucleus of amygdala (ACe). (A) Control. (B) Acute nicotine. (C) Acute diazepam. (D) Diazepam + nicotine. For details of the treatments see Table 1.

of 10 mg/kg IP elevates plasma corticosterone levels (25). On the basis of the present results one cannot say whether diazepam administration and stressful stimuli act on same neuronal targets. Additional experiments including rats exposed to stress are needed to clarify this point.

Diazepam, when administered prior to nicotine, did not prevent the nicotine-induced Fos expression in any of the brain areas studied. Furthermore, we found no significant interactions between acute effects of diazepam and nicotine on Fos IS, although nicotine tended to antagonize the diaze-

pam-induced elevation of Fos IS in ACe. Lack of interactions on Fos IS between diazepam and nicotine suggests that these drugs activate different sets of neurons in these brain areas. As discussed above, nicotine activates in pcPVN neurons mediating ACTH secretion and in SON both vasopressinergic and oxytocinergic neurons (21). At present, it is not clear which sets of neurons diazepam activates in these brain areas. Further, previous studies indicate no relationship between benzodiazepine binding site density and the extent to which diazepam decreased stress-induced c-*fos* expression (4).

Taken together, in our experiment nicotine induced Fos IS in both the central visual structures (MT, IP) and in PVN and SON, which are stress-related brain areas. Diazepam induced Fos IS in all stress-related areas studied (PVN, SON, ACe), but not in the central visual structures. No significant interactions were found between the acute effects of diazepam and nicotine on Fos expression, suggesting that these drugs activate different sets of neurons within the stress-related brain areas.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. Joe Herbert, Department of Anatomy, University of Cambridge, UK, and Dr. Pertti Panula, Department of Anatomy, University of Helsinki, Finland, for valuable advice and help with setting up the method for studying Fos IS. This work was supported by grants from the Finnish Cultural Foundation, the University of Helsinki and the Research Council for Health of the Academy of Finland.

REFERENCES

1. Amstrong, M. E. Cholinergic innervation of vasopressin-containing neurons in the supraoptic nucleus of the rat. In: Schrier, R. W., ed. Vasopressin. New York: Raven Press; 1985: 353-360.
2. Andersson, K.; Siegel, R.; Fuxe, K.; Eneroth, P. Intravenous injections of nicotine induce very rapid and discrete reductions of hypothalamic catecholamine levels associated with increases of ACTH, vasopressin and prolactin secretion. Acta Physiol. Scand. 118:35-40; 1983.
3. Arnold, F. J. L.; De Lucas Bueno, M.; Shiers, H.; Hancock, D. C.; Evan, G. I.; Herbert, J. Expression of c-*fos* in regions of the basal limbic forebrain following intracerebroventricular corticotropin-releasing factor in unstressed or stressed male rats. Neuroscience 51:377-390; 1992.
4. Beck, C. H.; Fibiger, H. C. Conditioned fear-induced changes in behavior and in the expression of the immediate early gene c-*fos*: With and without diazepam pretreatment. J. Neurosci. 15(1 Pt. 2):709-720; 1995.
5. Brown, M. R.; Gray, T. S. Peptide injections into the amygdala of conscious rats: Effects on blood pressure, heart rate and plasma catecholamines. Regul. Pept. 21:95-106; 1988.
6. Bullitt, E. Expression of c-*fos*-like protein as a marker for neuronal activity following noxious stimulation in the rat. J. Comp. Neurol. 296:517-530; 1990.
7. Campeau, S.; Hayward, M. D.; Hope, B. T.; Rosen, J. B.; Nestler, E. J.; Davis, M. Induction of the c-*fos* proto-oncogene in rat amygdala during unconditioned and conditioned fear. Brain Res. 565:349-352; 1991.
8. Ceccatelli, S.; Villar, M. J.; Goldstein, M.; Hökfelt, T. Expression of c-*fos* immunoreactivity in transmitter-characterized neurons after stress. Proc. Natl. Acad. Sci. USA 86:9569-9573; 1989.
9. Clarke, P. B. S.; Schwartz, R. D.; Paul, S. M.; Pert, C. B.; Pert, A. Nicotinic binding in brain—Autoradiographic comparison of [^{3}H]-acetylcholine, [^{3}H]-nicotine and [^{125}I]-a-bungarotoxin. J. Neurosci. 5:1307-1315; 1985.
10. Curran, T.; Franza, B. R. Fos and Jun: The AP-1 connection. Cell 55:395-397; 1988.
11. Curran, T.; Morgan, J. Superinduction of c-*fos* by nerve growth factor in the presence of peripherally active benzodiazepines. Science 229:1265-1268; 1985.
12. Deutch, A. Y.; Lee, M. C.; Gillham, M. H.; Cameron, D. A.; Goldstein, M.; Iadarola, M. J. Stress selectively increases fos protein in dopamine neurons innervating the prefrontal cortex. Cereb. Cortex 1:273-292; 1991.
13. Dragunow, M.; Faull, R. The use of c-*fos* as a metabolic marker in neuronal pathway tracing. J. Neurosci. Methods 29:261-265; 1989.
14. Dunn, J. D.; Whitener, J. Plasma corticosterone responses to electrical stimulation of the amygdaloid complex: Cytoarchitectural specificity. Neuroendocrinology 42:211-217; 1986.
15. Hayhow, W. R.; Webb, C.; Jervie, A. The accessory optic fiber system in the rat. J. Comp. Neurol. 115:187-215; 1960.
16. Honkaniemi, J.; Kainu, T.; Ceccatelli, S.; Rechardt, L.; Hökfelt, T.; Pelto-Huikko, M. Fos and Jun in rat central amygdaloid nucleus and paraventricular nucleus after stress. Neuroreport 3: 849-852; 1992.
17. Hunt, S. P.; Pini, A.; Evan, G. Induction of c-*fos*-like protein in spinal cord neurons following sensory stimulation. Nature 328: 632-634; 1987.
18. Ichikawa, T.; Hirata, Y. Organization of choline acetyltransferase-containing structures in the forebrain of the rat. J. Neurosci. 6:281-292; 1986.
19. London, E. D.; Connolly, R. J.; Szikszay, M.; Wamsley, J. K.; Dam, M. Effects of nicotine on local cerebral glucose utilization in the rat. J. Neurosci. 8:3920-3928; 1988.
20. London, E. D.; Waller, S. B.; Wamsley, J. K. Autoradiographic localization of [^{3}H]-nicotine binding sites in the rat brain. Neurosci. Lett. 53:179-184; 1985.
21. Matta, S. G.; Foster C. A.; Sharp B. M. Nicotine stimulates the expression of c-*fos* protein in the parvocellular paraventricular nucleus and brainstem catecholaminergic regions. Endocrinology 132(5):2149-2156; 1993.
22. Moore, R. Y.; Bloom, F. E. Central catecholamine neuron systems: Anatomy and physiology of the norepinephrine and epinephrine systems. Annu. Rev. Neurosci. 2:113-168; 1979.
23. Pang, Y.; Kiba, H.; Jayaraman, A. Acute nicotine injections induce c-*fos* mostly in nondopaminergic neurons of the midrain of the rat. Mol. Brain. Res. 20:162-170; 1993.
24. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1986.
25. Peričić, D.; Lacić, N.; Manev, H. Effect of diazepam on plasma corticosterone levels. Psychopharmacology 83:79-81; 1984.
26. Prouxl, L.; Giguere, V.; Levefre, G.; Labrie, F. Interactions between catecholamines, CRF and vasopressin in the control of ACTH secretion in the rat. In: Usdin, E.; Kvetnansky, R.; Axelrod, J., eds. Stress: The role of catecholamines and other neurotransmitters. Vol. 1. New York: Gordon and Breach Sci. Publ.; 1984:211-224.
27. Ren, T.; Sagar, S. M. Induction of c-*fos* immunostaining in the rat brain after the systemic administration of nicotine. Brain Res. Bull. 29:589-597; 1992.
28. Robertson, G.; Herrera, D.; Dragunow, M., Robertson, H. L-DOPA activates c-*fos* in the striatum ipsilateral to a 6-hydroxydopamine lesion of the substantia nigra. Eur. J. Pharmacol. 159:99-100; 1989.
29. Sagar, S. M.; Sharp, F. R.; Curran, T. Expression of c-*fos* protein in brain: Metabolic mapping at the cellular level. Science 240: 1328-1331; 1988.
30. Semba, K.; Fibiger, H. C. Organization of central cholinergic system. In: Nordberg, A.; Fuxe, K.; Holmstedt, B.; Sundwall, A., eds. Progress in brain research. Vol. 79. Amsterdam: Elsevier; 1989:37-63.
31. Sharp, B. M.; Beyer, S. H.; McAllen, K. M.; Hart, D.; Matta, S. G. Induction and desensitization of the c-*fos* mRNA response to nicotine in rat brain. Mol. Cell. Neurosci. 4:199-208; 1993.

32. Sharp, F. R.; Sagar, S. M.; Hicks, K.; Lowenstein, D.; Hisanaga, K. c-*fos* mRNA, Fos and Fos-related antigen induction by hypertonic saline and stress. J. Neurosci. 11(8):2321–2331; 1991.
33. Straaten, F.-V.; Muller, R.; Curran, T.; Beveren, C.-V., Verma, I. M. Complete nucleotide sequence of a human c-onc-gene: Deduced amino acid sequence of the human c-*fos* protein. Proc. Natl. Acad. Sci. USA 80:3183–3187; 1983.
34. Vale, W.; Spiess, J.; Rivier, C.; Rivier, J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213:1394–1397; 1981.
35. Wada, E.; Wada, K.; Boulter, J.; Deneris, E. F.: Heineman, S.; Patrick, J.; Swanson, L. W. Distribution of $Alpha_2$, $Alpha_3$, $Alpha_4$ and $Beta_2$ neuronal nicotinic receptor subunit mRNAs in the central nervous system: A hybridization histochemical study in the rat. J. Comp. Neurol. 284:341–335; 1989.
36. Woolf, N. J.; Butcher, L. L. Cholinergic systems in the rat brain: 2. Projections to the interpeduncular nucleus. Brain Res. Bull. 14:63–83; 1985.
37. Zbuzek, V. K.; Zbuzek, V. Effect of chronic nicotine treatment and its withdrawal on the vasopressinergic system in rats. J. Neuroendocrinol. 3:107–112; 1991.