Pharmacology Biochemistry & Behavior, Vol. 22, pp. 317–325, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Effects of Nicotine on β-Endorphin, αMSH, and ACTH Secretion by Isolated Perfused Mouse Brains and Pituitary Glands, *in Vitro*

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Received 29 May 1984

MARTY, M. A., V. G. ERWIN, K. CORNELL AND J. M. ZGOMBICK. *Effects of nicotine on β-endorphin, αMSH, and ACTH secretion by isolated perfused mouse brains and pituitary glands*, in vitro. PHARMACOL BIOCHEM BEHAV 22(2) 317–325, 1985.—The effects of nicotine on secretion of the pituitary peptides β-endorphin, αMSH, and ACTH were studied using the isolated perfused mouse brain (IPMB) and isolated superfused pituitaries of C3H mice. Nicotine (6.1 μM) stimulated secretion of β-endorphin immunoreactivity from C3H IPMB approximately twofold. Secretion of αMSH immunoreactivity was stimulated approximately two- and sixfold by 6.1 μM and 12.2 μM nicotine, respectively. However, nicotine (6.1 μM) had no direct effect on the secretion of β-endorphin, αMSH, or ACTH immunoreactivities from the isolated superfused pituitaries. The data suggest nicotine acts in the brain to stimulate pituitary secretion of αMSH and β-endorphin. Electrocorticographic (ECoG) activity of the IPMB was monitored. Nicotine induced characteristic ECoG changes including a reduction of input voltage, a biphasic response of rapid desynchronization followed by prolonged synchronization, and seizure at high doses (12.2 μM).

Nicotine β-Endorphin αMSH ACTH Pituitary Isolated perfused mouse brain

NICOTINE-INDUCED changes in the secretion of adenohypophyseal hormones have been documented by several investigators. The increase in plasma corticosteroid levels following nicotine administration in laboratory animals [7] and cigarette smoking in humans [42] may be attributed to nicotine-induced release of ACTH by the pituitary [10]. Other hormones whose release *in vivo* is affected by nicotine include prolactin [42], growth hormone [42], vasopressin [4], and LH, FSH, and TSH [2,3].

β-Endorphin, αMSH, and ACTH are all part of a large precursor glycoprotein, proopiomelanocortin (POMC) which is processed in the pituitary to several peptide products. Many studies have demonstrated the co-release of ACTH and β-endorphin from the pituitary gland under a variety of conditions [1,18]. Meunier and colleagues [30] have recently documented the co-release of αMSH and ACTH from pars intermedia cells in response to certain stimuli. Therefore it was of interest to determine the effect of nicotine on secretion of β-endorphin and αMSH. The present study utilized the isolated perfused mouse brain (IPMB) which has been characterized as a viable system for neurochemical and neuropharmacological studies [37]. The advantage of this particular methodology is the elimination of interfering feedback regulation and peripheral metabolic degradation of both the neuropeptides and nicotine. The mouse brain was used because of availability of several strains of mice which differ in behavioral and physiological responses to nicotine [29]. The IPMB will allow comparison among these strains of mice.

The effect of nicotine on secretion of β-endorphin, αMSH, and ACTH by isolated superfused pituitary fragments was also studied. Comparison of experimental results with these two *in vitro* systems will facilitate description of the site and mechanism of action of nicotine.

METHOD

Animals

C3H/2Ibg mice were bred at the Institute for Behavioral Genetics, Boulder, CO. After weaning, animals were housed at the School of Pharmacy Animal Facility, University of Colorado, Boulder, and maintained on a 12L/12D cycle. Food (Wayne Rodent Blox) and water were provided ad lib. Experiments were performed with male mice, 60 to 100 days of age.

Brain Perfusion Procedure

Surgical preparation of the IPMB has been described in detail by Andjus *et al.* [5]. The preparation has been characterized [37] using a washed bovine erythrocyte suspension

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TABLE 1
ANTISERA CROSS-REACTIVITY SUMMARY

Antiserum	Peptide		
	β-Endorphin	αMSH	ACTH
β-Endorphin	1	0	0.0007
αMSH	0.0005	1	0.0008
ACTH (West)*	0.004	0.004	1

*According to specifications [31].

according to the electrophysiological, morphological, and biochemical criteria suggested by Woods *et al.* [43]. Spontaneous electrocortical activity is one of the most sensitive indicators of the viability of the preparation; it becomes isoelectric as lactate levels rise. Immediately prior to surgical preparation for perfusion, animals were anesthetized with urethane (1.5 g/kg, IP), and bipolar platinum electrodes were placed on the temporal and frontal surfaces of the cerebral cortex and fixed in place with carboxylate dental cement. As soon as the cement had hardened (approximately 20 min), the internal carotid arteries were cannulated and the brains were perfused with an artificial blood containing perfluorotributylamine as an oxygen carrier at 37°C (FC-43 Emulsion; Green Cross Corp., Osaka, Japan). Sloviter and Kamimoto [35] have demonstrated that isolated rat brains perfused with such emulsions retain electrical activity as well as or better than brains perfused with an erythrocyte suspension. The perfused brains were placed in a thermoregulated plexiglass chamber and maintained at a temperature of 37°C.

Electrocorticographic activity was measured by connecting the cerebral electrodes to a Grass Model 7P5B AC EEG pre-amplifier and a Model 7DAF driver amplifier with a 0.1-sec time constant and a 75-Hz half amplitude cutoff. A Grass Model FP10 summating integrator was used to provide a running record of "total accumulated area under the curve" of input voltage plotted against time. Control electrocortical activity, in absence of nicotine, was compared with activity in the presence of nicotine at various times and doses. Perfusion fluid was collected in plastic tubes on ice containing a total of 5 mg EDTA plus 0.5 KIUs of Aprotinin (Trasylol; FBA Pharmaceuticals, New York, NY) per ml of perfusion fluid collected. The samples were frozen and stored at −20°C until analyzed for β-endorphin and α-MSH content by RIA (approximately 3 weeks).

Pituitary Superfusion Procedure

Animals were sacrificed by decapitation, the brains removed, and the pituitary glands isolated. Posterior pituitary was carefully detached and the remaining anterior and intermediate lobe placed in a superfusion chamber. The chamber consisted of a plastic pipette tip containing a glass wool plug. Oxygenated Medium 199 with Hank's salts and without sodium bicarbonate maintained at 37°C (GIBCO Laboratories, Grand Island, NY) was pumped through each chamber with a Gilson Minipuls 2 (Middleton, WI) peristaltic pump at a flow rate of 0.15 ml/min. The chambers were placed in an incubator at 37°C. Incubation procedures were adapted after those of Pokras and Tabakoff [32] who determined viability of the preparation and validity of the procedure for study of prolactin secretion. A 2-hr equilibration period was followed by three collection periods of 45 min duration. The first period (A) was a control period. The second period (B) was a treatment period wherein nicotine, K^+, Ca^{++}, or corticotropin releasing factor (CRF) (ovine, Peninsula Laboratories, Belmont, CA) was added. Period C served as a final control period. The superfusion fluid was collected on ice into tubes containing 20 KIU Traysolol and stored at −20°C until RIA analysis for ACTH, αMSH, and β-endorphin. Prolonged storage (greater than 3 weeks) was avoided.

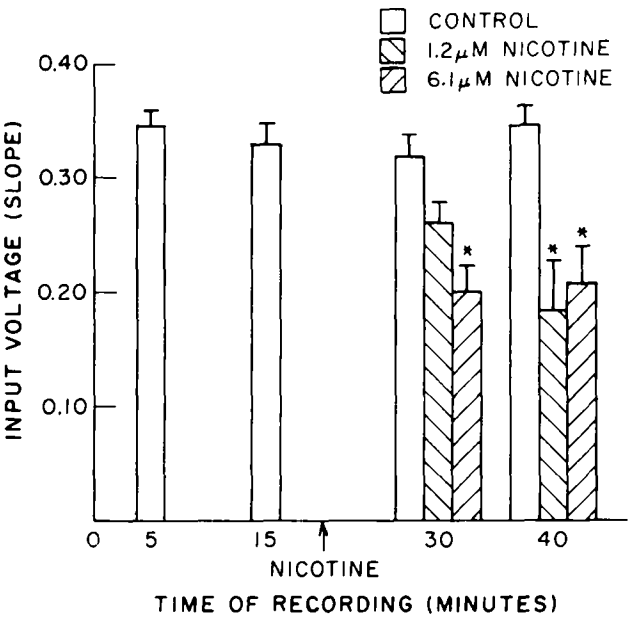

FIG. 1. Dose-dependent effect of nicotine on electrocortical activity of the IPMB. Doses of 1.2 μM and 6.1 μM nicotine were added to the perfusion fluid at 20 min. *$p<0.05$.

Radioimmunoassay

Rabbit antisera to β-endorphin (mid-portion directed) and αMSH (C-terminal directed) were kindly provided by Dr. Robert Eskay, NIAAA. Rabbit antiserum to ACTH, generated by Dr. C. D. West, was generously provided by the National Pituitary Agency, NIAMDD, U.S. Dept. of Health and Human Services. The cross-reactivities of these antisera are summarized in Table 1. The ligand at which the antibody is directed is assigned one unit of reactivity. The other ligands cross-reacted from 0.07% to 0.4%. This level is considered negligible. Synthetic human ACTH 1–39 (donated to NIAMDD by Ciba-Geigy), β-endorphin (human), and αMSH (Peninsula Laboratories, Belmont, CA) were iodinated by the chloramine-T procedure of Hunter and Greenwood [22]. Iodinated ligand was purified on a BSA-pretreated Sephadex G10 column (10-ml bed volume), followed by chromatography on a 40×1 cm BSA-pretreated Sephadex G50 column. Phosphate-buffered (0.01 M) saline was used to elute the peptides from the columns. The purified ligand was stored at −70°C.

Each sample of IPMB perfusion fluid was divided into two and following the addition of Triton X100 (final concentration, 1%) to one portion, both were centrifuged at 22,000 g × 45′ to pellet the emulsified perfluorocarbon. Triton X100

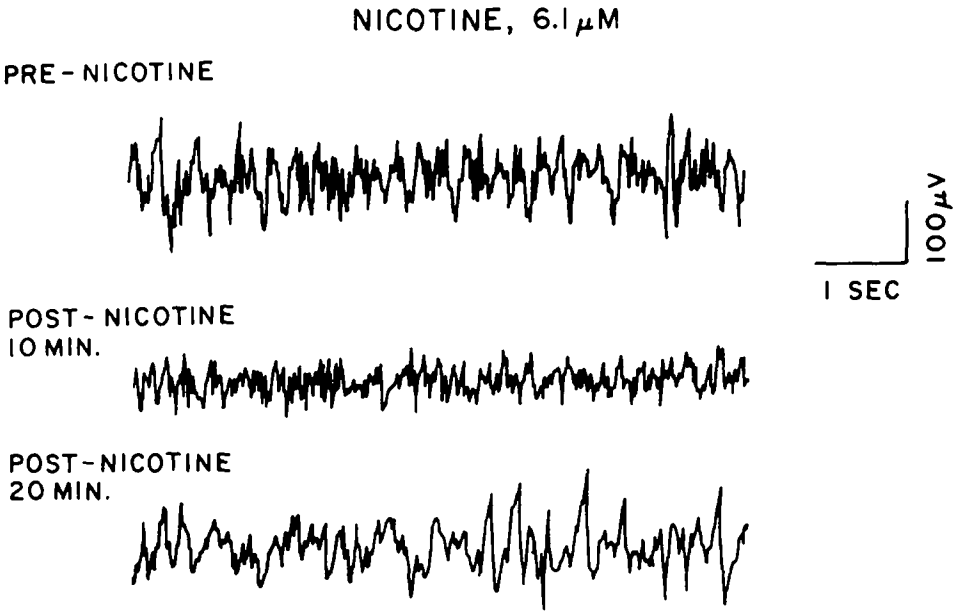

FIG. 2. Typical electrocorticographic record following 6.1 μM nicotine. Note the biphasic response with desynchronization at 10 min post nicotine and synchronization at 20 min post nicotine.

was necessary to prevent β-endorphin from binding to the perfluorocarbon. Recoveries of αMSH and β-endorphin in the supernatant fluid were 80±2% and 83±3%, respectively. The supernatant fluid could be directly assayed for αMSH and β-endorphin.

The pituitary superfusion samples could be analyzed directly for αMSH, β-endorphin, and ACTH by RIA.

RESULTS

Electrocorticographic Activity

The IPMB was used to determine the effects of steady-state concentrations of nicotine on electrocorticographic (ECoG) activity of perfused C3H mouse brain. Data from right and left bipolar electrode recordings (Fig. 1) show a concentration-dependent reduction of input voltage at 10 min post nicotine. Ten minutes following addition of nicotine (6.1 μM), a 36% reduction of input voltage from control values, $p<0.05$ (n=20) was observed. IPMB preparations which maintained ECoG viability for 1 hr typically displayed a biphasic response to nicotine, with a rapidly occurring desynchronization followed by prolonged synchronization (Fig. 2). At a concentration of 12.2 μM, nicotine produced a brief desynchronization followed by seizures (Fig. 3). A distinct theta rhythm was then present followed by a second seizure episode which rapidly led to spiking and a silent ECoG.

Secretion of β-Endorphin and αMSH by IPMB

Both αMSH and β-endorphin immunoreactivities (αMSH-ir and β-endorphin-ir) were detected in perfusion fluid from the IPMB (Tables 2A and 2B and Figs. 4 and 5). The samples collected from 10 to 20 min after the start of the perfusion were used as basal secretion rates for each group. Nicotine or saline was added to the perfusion fluid at 20 min and samples were collected for 10-min intervals. Collections began at 25 min, 5 min after addition of nicotine to the perfusion fluid reservoir, allowing time for nicotine to reach the brain.

Nicotine produced a transient stimulation of β-endorphin and αMSH secretion from the IPMB. This increased secretion was observed during the 25–35 min perfusion period (Tables 2A and 2B, Figs. 4 and 5) just after the addition of nicotine to the perfusion fluid. Since our experimental design involves both between-subjects (nicotine dose) and within-subjects (repeated measures over time) factors, a mixed-design analysis of variance was used to examine the data [26] followed by Scheffé tests. Mixed-design analysis of variance reveals a significant effect of nicotine dose on both αMSH-ir secretion, $F(6,46)=3.408$, $p<0.05$, and on β-endorphin-ir secretion, $F(4,24)=3.0864$, $p<0.05$. The increase in β-endorphin-ir secretion is evident at a nicotine concentration of 6.1 μM. This concentration of nicotine produced a β-endorphin-ir secretion rate 1.75-fold higher than in the corresponding control period, $p<0.05$, Scheffé test (Table 2A, Fig. 4). As the nicotine concentration of the perfusion fluid was increased from 1.2 μM to 12.2 μM, the stimulation of αMSH-ir secretion also increased. Nicotine produced a 2.5-fold and a 5.7-fold rise in αMSH-ir secretion at 6.1 μM and 12.2 μM, respectively, relative to the preceding control periods, $p<0.05$, Scheffé test (Table 2B and Fig. 5).

In order to evaluate any direct effect of nicotine on the pituitary, we investigated the effects of nicotine on neuropeptide secretion by isolated superfused pituitary fragments. To verify that the C3H mouse pituitary fragments were viable, we examined the secretion of ACTH immunoreactivity (ACTH-ir) in response to high K^+, high Ca^{++}, and CRF. Chambers containing anterior and intermediate lobe fragments were superfused as described in Method. During the B period, three chambers received 60 mM K^+ in M199, three received 8 mM Ca^{++} in M199, and three were perfused with 10^{-9} M CRF in M199. Control chambers were superfused with M199. Each of the sec-

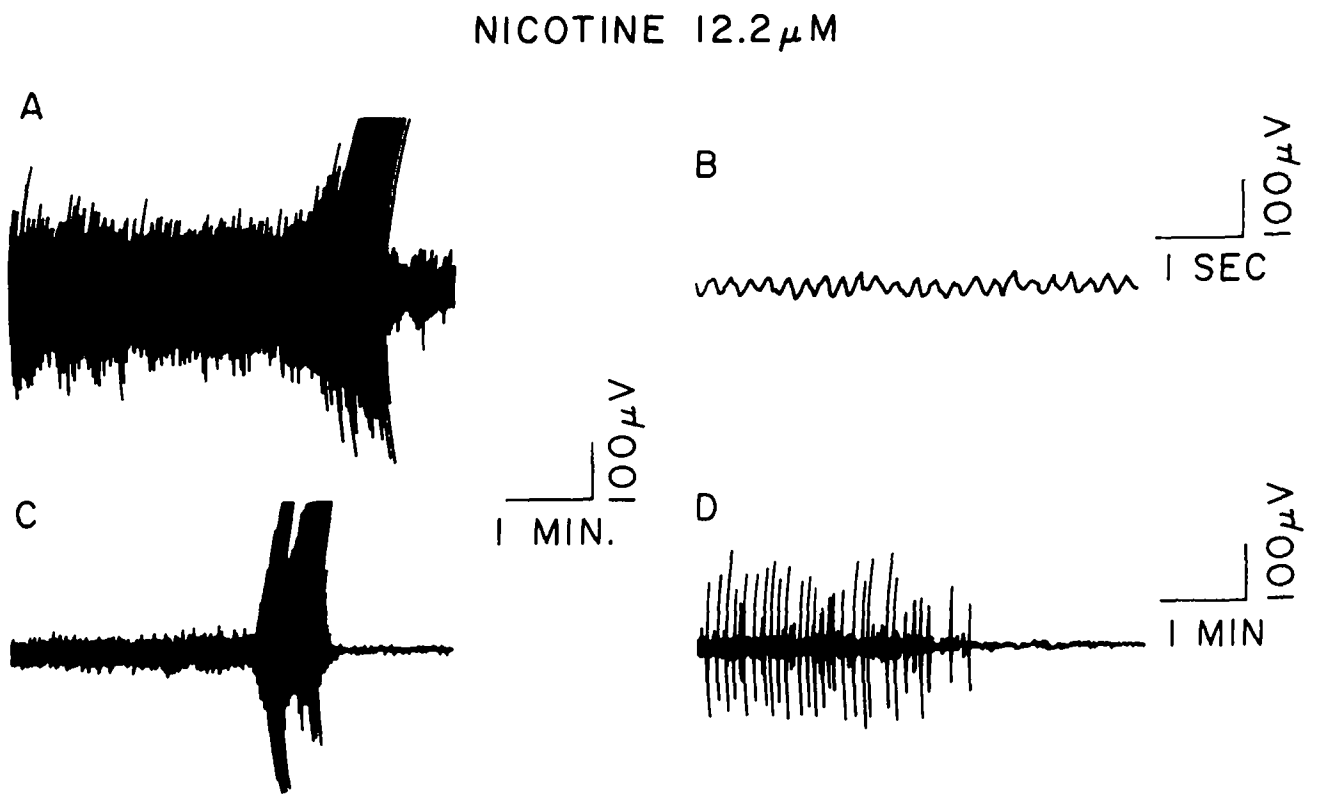

FIG. 3. Effects of 12.2 μM nicotine on the ECoG of the C3H IPMB. A. Within 2 min, a grand mal seizure occurs after a slight desynchronization. B. Rhythmic theta activity recorded from cortical bipolar electrode 6 min post nicotine. C. Second seizure episode occurring 9 min after the first seizure. D. Spike activity leading to electrical silence following the second set of seizures.

TABLE 2A

β-ENDORPHIN SECRETION FROM THE IPMB

		β-Endorphin (pg/min/brain ± SE) Collection Time (min)*				
	N†	10–20	N	25–35	N	35–45
No nicotine	4	591 ± 167	4	430 ± 93	4	503 ± 68
1.2 μM Nicotine	6	383 ± 94	6	402 ± 82	5	318 ± 77
6.1 μM Nicotine	5	619 ± 45	5	1089 ± 278‡	5	626 ± 162

*Collection Time=the time in minutes after the start of perfusion during which the sample was collected.

†N=number of IPMBs per treatment group.

‡Nicotine dose has a significant effect on β-endorphin secretion relative to control periods, mixed-design analysis of variance, $F(4,24)=3.0864$, $p<0.05$. At 6.1 μM nicotine, β-endorphin secretion rate was 1.75-fold greater during the 25–35 min collection time than in the preceding control period, $p<0.05$, Scheffé test.

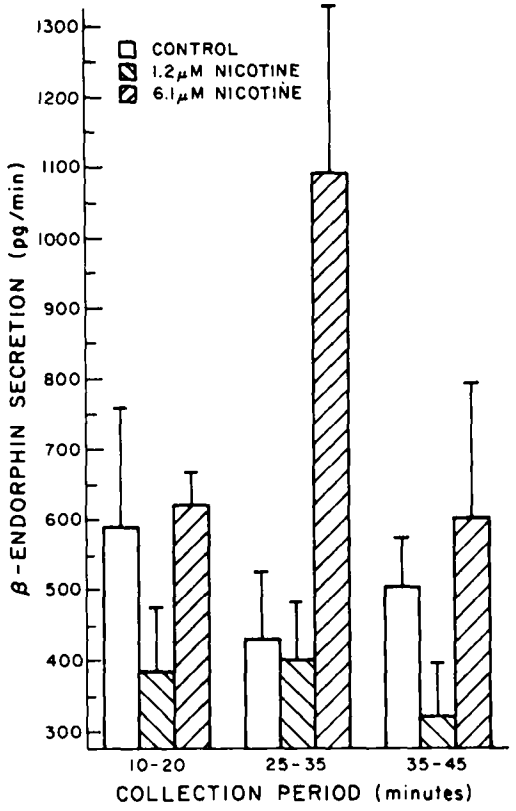

FIG. 4. Effect of nicotine on β-endorphin-ir secretion from the IPMB. Control samples were collected from 10–20 min after the start of perfusion. At 20 min, nicotine (1.2 or 6.1 μM) or saline was added to the perfusion fluid reservoir. Sample collection was resumed at 25 min, allowing 5 min for nicotine to reach the brain. Ten-minute samples were collected. Data are tabulated in Table 2A. Nicotine dose had a significant effect on β-endorphin-ir secretion at 25–35 min relative to the preceding 10–20 min control period, mixed-design analysis of variance, $F(4,24)=3.086$, $p<0.05$.

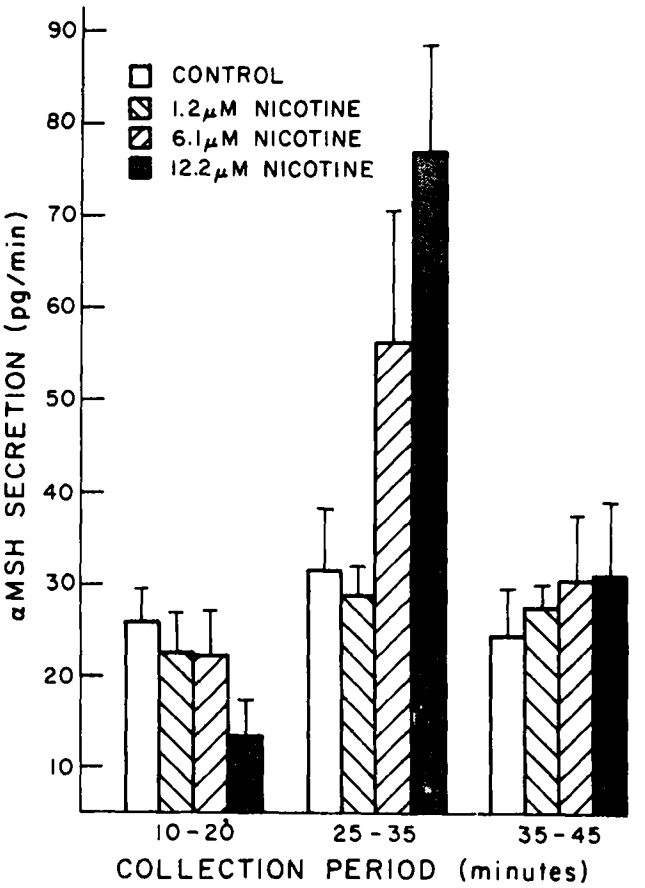

FIG. 5. Effect of nicotine on αMSH-ir secretion from the IPMB. Samples were collected as described in Fig. 4. Three concentrations of nicotine were used, 1.2 μM, 6.1 μM, and 12.2 μM. Data are tabulated in Table 2B. Nicotine dose had a significant effect on αMSH-ir secretion at 25–35 min relative to the preceding 10–20 min control period, mixed-design analysis of variance, $F(6,46)=3.408$, $p<0.05$.

TABLE 2B

α-MSH SECRETION FROM THE IPMB

		αMSH (pg/min/brain±SE) Collection Time (min)*				
	N†	10–20	N	25–35	N	35–45
No nicotine	9	26.7 ± 3.4	9	31.5 ± 6.6	9	24.5 ± 5.3
1.2 μM Nicotine	5	22.4 ± 4.3	5	28.7 ± 2.9	5	27.5 ± 2.6
6.1 μM Nicotine	11	22.1 ± 5.0	11	56.1 ± 14.4‡	10	31.4 ± 6.2
12.2 μM Nicotine	3	13.4 ± 3.8	3	76.7 ± 11.9‡	2	31.9 ± 6.9

*Collection Time=the time in minutes after the start of perfusion during which the sample was collected.

†N=number of IPMBs per treatment group.

‡Nicotine dose has a significant effect on αMSH secretion relative to control periods, mixed-design analysis of variance, $F(6,46)=3.408$, $p<0.05$. At 6.1 μM and 12.2 μM nicotine, αMSH was 2.5- and 5.7-fold greater during the 25–35 min collection time than in the preceding control periods, $p<0.05$, Scheffé test.

TABLE 3

SECRETION OF ACTH BY SUPERFUSED C3H MOUSE PITUITARIES: STIMULATION BY HIGH [K^+] (60 mM), HIGH [Ca^{++}] (8 mM), AND CRF (10^{-9} M)

	Superfusion Period (45 min)				
	A		B		
N*	Medium	pg ACTH/hr/2 pit†	Medium	pg ACTH/hr/2 pit†	B/A
3	M199	3330 ± 286‡	High K^+	5000 ± 67§	1.5
3	M199	2940 ± 793	High Ca^{++}	5208 ± 997§	1.8
3	M199	2230 ± 304	CRF	3042 ± 72§	1.4
3	M199	2075 ± 270	M199	1864 ± 211	0.9

*N=Number of chambers perfused.
†pit=Fragmented pituitaries.
‡Standard deviation.
§Statistically different from their respective A periods, $p<0.05$, Student's t test.

TABLE 4

EFFECT OF NICOTINE ON NEUROPEPTIDE SECRETION BY SUPERFUSED C3H MOUSE PITUITARIES

	Superfusion		β-Endorphin		αMSH		ACTH	
Experiment	Period*	Medium†	N‡	B/A	N	B/A	N	B/A
1	A	M199						
	B	M199	10	0.70 ± 0.34§	11	0.59 ± 0.17	3	0.98 ± 0.07
	C	M199						
2	A	M199						
	B	Nicotine	14	0.57 ± 0.15	14	0.53 ± 0.22	3	1.19 ± 0.17
	C	M199						
3	A	CRF						
	B	CRF	7	1.12 ± 0.41	7	1.12 ± 0.44	3	0.95 ± 0.19
	C	CRF						
4.	A	CRF						
	B	CRF/Nicotine	7	0.71 ± 0.25	7	0.70 ± 0.35	3	0.87 ± 0.16
	C	CRF						

*Each period 45 minutes long, see text.
†Nicotine concentration=6.1 μM in M199; CRF concentration=10^{-9} M in M199.
‡N=Number of chambers superfused. Each chamber had pituitary fragments from 2 or 3 pituitaries.
§Standard deviation.

retagogues stimulated the secretion of ACTH-ir by the anterior and intermediate lobe fragments (Table 3). The amount of ACTH-ir secreted (pg ACTH/hr/2 pituitaries) is significantly greater in the B period than in the A period with all three treatments (Student's t test, $p<0.05$). The B/A ratios show high K^+ concentration produced a 1.5-fold stimulation of ACTH-ir release, while high Ca^{++} produced a 1.8-fold and CRF a 1.4-fold stimulation of ACTH-ir release. The control chambers showed a slight decrease in secretion in the B period. The data indicate the pituitary fragments are viable and respond to augmented levels of K^+ and Ca^{++} and to CRF.

Nicotine at a concentration of 6.1 μM in M199 had no effect on the secretion of ACTH-ir, αMSH-ir, or β-endorphin-ir by the superfused anterior and intermediate lobe pituitary fragments (Table 4). The data in the table are expressed as a ratio of secretion in the B period relative to the A period. A typical secretion rate for β-endorphin-ir in the A period was 55 ng β-endorphin-ir per hr per anterior and intermediate lobe (range=7 to 90). An average secretion rate for αMSH-ir in the A period was 8 ng/hr per anterior and intermediate lobe (range=4 to 20). There are no significant differences between B/A in Experiments 1 and 2 (Table 4) whether the pituitaries were superfused with M199 for all three periods (1) or with 6.1 μM nicotine in M199 for period B (2). Similarly, when CRF was present in the superfusion medium (3 and 4), nicotine had no significant effect on secretion of β-endorphin-ir, αMSH-ir, or ACTH-ir. The data suggest that nicotine at 6.1 μM does not directly affect anterior and intermediate lobe secretion of these three neuropeptides. Rather, the IPMB data suggest that nicotine's effects on αMSH-ir and β-endorphin-ir secretion are mediated through brain structures such as the hypothalamus.

DISCUSSION

Electrocortical Activity

The primary purpose for monitoring electrical activity in the IPMB is to verify the viability of the preparation under the varying conditions used in these studies. It is interesting to note that the effects of the various concentrations of nicotine on the electrocortical activity of the IPMB parallel those reported by Longo *et al.* [28] in pre-pontine brainstem-transected rabbit, with small doses (0.02–0.05 mg/kg) resulting in activation of the EEG and larger doses (1–2 mg/kg) provoking seizure followed by electrical silence. The pattern of desynchronization at low doses, which would indicate a state of behavioral arousal, and seizure at a higher dose correlates well with data reported by Marks *et al.* [29] on locomotor activity of C3H mice at varying doses of nicotine. These reports extend previous observations [14] on reduction of ECoG amplitude by nicotine in C3H mice.

An important finding by Yamamoto *et al.* [44] has been that the limbic system is also affected by nicotine, since theta waves appear in the hippocampus before the initial cortical change and are present even when this initial effect is blocked by destruction of the reticulocortical pathways. Weiss and Fifkova [41] demonstrated that posterior placement of electrodes on the cortex adequately records hippocampal rhythmic activity in mice due to volume conduction through the layer of neocortex that overlies the hippocampus. The IPMB with electrodes on the cortical surface demonstrated theta activity following a 2 mg/kg dose of nicotine indicating a functional limbic system.

Domino [13] observed a biphasic electrical response to low doses of nicotine and a tachyphylaxis to repeated doses of nicotine given 10 min apart. The IPMB appeared to demonstrate these effects with desynchronization followed by synchronization in the presence of steady-state levels of nicotine. It is evident from the present studies that the effects of nicotine on cerebral electrical activity of the IPMB are similar to those observed using whole animals and are due to a direct effect of nicotine upon the CNS.

Secretion of αMSH-ir and β-Endorphin-ir by the IPMB

The IPMB responded to nicotine with an increased secretion of β-endorphin-ir and αMSH-ir. These two POMC-derived peptides are located primarily in the pituitary gland. Hypothalamus, thalamus, and other brain regions also contain αMSH and β-endorphin immunoreactivity [12, 15, 17, 25] but at levels approximately three orders of magnitude lower than those in the pituitary gland. The high secretion rates observed in this study implicate the pituitary as the source of these neuropeptides. αMSH is the major POMC peptide in the intermediate lobe. β-Endorphin is located in both the intermediate and anterior lobes of the pituitary but is in higher concentration in the intermediate lobe cells [16, 20, 24]. Although secretion of hormones by anterior lobe cells and intermediate lobe cells might be regulated by different mechanisms [33,38], nicotine may affect secretion from both lobes. Nicotine has been shown to elevate ACTH levels in rats [4,10] and humans [42]. The concomitant secretion of β-endorphin and ACTH from the anterior pituitary has been documented by many investigators [1,18] in response to a variety of stimuli. If nicotine can stimulate secretion of all three peptides, it must be able to exert influence over both the intermediate and anterior pituitary lobes. Nicotine did not stimulate β-endorphin-ir, αMSH-ir, or ACTH-ir release from isolated anterior and intermediate lobes in the present study. This suggests that nicotine is acting in the brain to influence pituitary secretion of these POMC-derived neuropeptides. The hypothalamic release of CRF is under cholinergic control. Both nicotinic and muscarinic receptors are involved, but they appear to be primarily nicotinic [21,40]. Thus nicotine may act to increase anterior pituitary secretion of ACTH and β-endorphin through a stimulation of CRF release in the hypothalamus. CRF has been shown to stimulate αMSH secretion from pars intermedia cells [30]. It is possible that nicotine-induced secretion of αMSH results from increased CRF release by the hypothalamus. However, it has also been reported that CRF is ineffective in stimulating αMSH secretion by the intermediate lobe [20].

Nicotine has been shown to increase hypothalamic dopamine and norepinephrine turnover with concomitant increases in plasma levels of the pituitary hormones, growth hormone, prolactin, and LH in rats [2,4]. Dopamine fibres originating in the arcuate nucleus of the hypothalamus tonically inhibit αMSH release from the intermediate lobe [36]. Stimulation of β2 adrenoceptors on the intermediate lobe by isoproterenol stimulates αMSH release [17]. Similarly, the β-adrenergic agonist isoprenoline stimulates secretion of β-endorphin from intermediate lobe *in vitro* while dopamine inhibits β-endorphin secretion *in vitro* [39]. It is conceivable that nicotine alterations in hypothalamic catecholamine activity [2,4] could result in an increase in αMSH and β-endorphin secretion from the intermediate lobe.

The nicotine-induced stimulation of α-MSH-ir and β-endorphin-ir release from the IPMB was transient with a peak at 25–35 min. Secretion in the 35–45 min collection periods had returned to control levels. Tachyphylaxis to the nicotine-induced changes in the ECoG has been noted, and rapid tolerance to other nicotine actions including depressant effect in rats and cardiovascular disturbances in smokers has been reported [23]. It is not known whether depletion of stores of the processed neuropeptides or some other mechanism accounts for the tachyphylaxis in αMSH-ir and β-endorphin-ir secretion observed in the present study.

A variety of behavioral and physiological responses to nicotine have been well documented [6, 8, 19, 29, 34]. αMSH and β-endorphin can produce behavioral and physiological responses similar to those seen with nicotine such as grooming behavior [9], arousal, antinociception [9,19], and hypothermia [11,27]. It is conceivable that some of nicotine's actions in both laboratory animals and man may be partially mediated through the release of POMC peptides.

In summary, the IPMB proved to be a useful model to study release of pituitary peptides in response to nicotine. The availability of selectively bred lines of mice, which differ in their responses to nicotine, will aid in elucidating roles of the POMC peptides in the variety of responses to nicotine.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Dr. Robert Pokras for his assistance with radioimmunoassay and the superfused pituitary system and Mr. Roger Little and Dr. James Wilson for their help with the analysis of variance. This work was supported in part by The Council for Tobacco Research Grant 1500R1 and by USPHS Grant AA03527. M. A. Marty was a NIDA postdoctoral trainee, USPHS Grant DA07043.

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