min^{-1}) shearing with the Silverson mixer before a similar change could be effected. This difference suggests a weaker degree of polymer-polymer interaction in the electrolyte-flocculated gels. Perhaps the increase in particle size and the irregularity of particle shape in the flocculated systems led to the weaker cohesion. A redispersion of the electrolyte-flocculated gel (produced by shaking) tended to retain fluidity longer than the original dispersion challenged by the electrolyte (Fig. 4), but the difference was not significant.

The conclusion is that aqueous coacervated gels of the polymers studied have inherent strong interactions that can only be broken down at high shear rates to produce liquid dispersions. Their stability depended on the presence of polymer cations. Addition of an electrolyte imparted a thixotropic property to the polymer dispersions. This flow property may be exploited in the formulation of muco-adhesive gels which are pourable upon shaking but revert to a gel consistency after administration.

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Effect of chronic administration of nicotine or cocaine on steroidogenesis in rat adrenocortical cells

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Abstract—Rats were implanted subcutaneously with osmotic mini-pumps containing either 0.9% NaCl, nicotine (1.5 or 4.5 mg kg^{-1} day^{-1}), or cocaine (30 mg kg^{-1} day^{-1}), for 14 days. Neither nicotine nor cocaine treatment significantly altered the maximal rate of steroidogenesis in adrenocortical cell preparations from the animals. However, pretreatment with cocaine increased the sensitivity of the preparation to stimulation by ACTH, the ED50 was 5 pM compared with 10 pM from control animals. Addition of nicotine or cocaine at concentrations up to 100 μM to adrenal cell suspensions from naive rats did not stimulate steroidogenesis or increase the sensitivity of cells to ACTH stimulation. These results suggest that the primary chronic effect of nicotine on steroidogenesis is exerted at the level of the hypothalamus and/or pituitary and not directly on adrenocortical cells. On the contrary, pretreatment with cocaine causes persistent changes in adrenocortical cells.

Nicotine has been shown to produce marked neuroendocrine changes in laboratory animals and man (Andersson et al 1982, 1988; Cam & Bassett 1983; Fuxe et al 1989). Its acute administration increases the plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone (Conte-Devolx et al 1981; Cam & Bassett 1983). Fuxe et al (1989) suggested that some endocrine related health problems result from nicotine derived from cigarette smoke. This effect may involve central nicotinic cholinergic receptors at the hypothalamic cholinergic neurons (Hillhouse et al 1975; Weidenfeld et al 1983). This is supported by the observation that nicotine stimulates the secretion of ACTH in a dose-dependent manner when applied directly to the hypothalamus, but not to the pituitary of an isolated perfused mouse-brain preparation (Marts et al 1985). Although most of the studies have been directed primarily towards examining the effects of nicotine on the hypothalamic-pituitary portion of the glucocorticoid regulatory system, nicotine may exert an acute direct effect at the level of the adrenocortical cells (Rubin & Warner 1975).

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Similarly to nicotine, cocaine is a central nervous system stimulant which interacts with several central monoaminergic neurons to exert its psychotropic effects (Gregler & Marks 1986; Hanson et al 1987; Ritz et al 1987). Acute intravenous administration of cocaine to rats has been shown to stimulate the release of ACTH, which can be abolished by the administration of corticotropin releasing factor (CRF) antiserum (Rivier & Vale 1987). This suggests that cocaine acts within the brain to release endogenous CRF. Direct stimulation of ACTH release by cocaine under conditions where CRF release was inhibited has also been reported (Moldow & Fischman 1987).

In light of the close interaction between the central nervous system and the endocrine system, stimulants such as nicotine and cocaine may have profound effects on the neuroendocrine system. Most studies that have examined their effects on the secretion of ACTH and corticosterone have involved systemic administration of drug. Little information is available about the direct effects of their continuous administration on the secretion of hormones at the level of the adrenocortical cell. We have examined the effects in the rat of continuous administration of nicotine or cocaine via subcutaneous implantation of an osmotic mini-pump containing the drug.

Materials and methods

Materials. Corticosterone, collagenase, DNase I, N^6, 2'-O-dibutyryl cAMP (bt$_2$cAMP), porcine ACTH, nicotine tartrate and cocaine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). ACTH was purified by ion-exchange (Yamashiro et al 1984) and reversed phase chromatography. Ham's F-12 medium was obtained from Gibco (Grand Island, NY). 20(R),22(R)-dihydroxycholesterol was a generous gift of Dr Nanette Orme-Johnson, Tufts University School of Medicine (Boston, MA). Alzet osmotic mini-pumps model 2002 were obtained from Alza Corp (Palo Alto, CA).

Animals. Male Sprague-Dawley rats (Sasco, Inc., Omaha, NE),

180–200 g, were housed in groups of 3 per cage in a light and temperature (23 ± 1°C) controlled environment with a 12 h light-dark cycle and allowed free access to food (Purina Lab. Chow, St. Louis, MO) and water. Animals were anaesthetized with an isoflurane/oxygen mixture and were implanted subcutaneously posterior to the shoulder with an Alzet osmotic mini-pump model 2002. Each treatment group contained five rats. These pumps were filled with either sterile 0·9% NaCl (saline), nicotine to give either 1·5 or 4·5 mg kg^{-1} day^{-1} or cocaine to give 30 mg kg^{-1} day^{-1}. Before implantation, each pump was primed overnight at 37°C in saline. The doses of nicotine or cocaine were calculated as the amount of free base provided by nicotine tartrate or cocaine hydrochloride dissolved in sterile saline. All chronic studies were conducted 14 days after pump implantation.

Preparation of rat adrenocortical cell suspensions. Rats were anaesthetized with halothane, killed by cervical dislocation, and the adrenal glands removed, trimmed of fat and weighed. Adrenocortical cell suspensions were prepared as described by Goverde et al (1980). Ham's F-12 medium with 2·5 mM Ca^{2+} was used for cell purification and incubation. Viable cell concentrations were determined by dye exclusion using erythrosin B (Phillips 1973).

Measurement of steroidogenesis. The steroidogenic properties of rat adrenocortical cells were determined by incubation of cell suspensions in 12 × 75 mm capped polypropylene tubes. Cells were incubated in Ham's F-12 medium containing 0·2% glucose and 0·5% bovine serum albumin under an atmosphere of 5% CO_2/95% air. The viable cell quantity was 10 000 cells/tube in a final volume of 0·5 mL after agonist addition. Incubations were carried out in an oscillating (30 cycles min^{-1}) water bath at 37°C for 60 min. Steroid production was terminated by addition of 1·0 mL dichloromethane to the cell suspension followed by mixing. Corticosterone was determined using a fluorometric assay

FIG. 2. Chronic nicotine treatment does not alter the steroidogenic responsiveness of adrenocortical cells to ACTH. Adrenocortical cell suspensions were prepared as in Fig. 1 from rats implanted with minipumps delivering saline (O), 1·5 mg kg^{-1} day^{-1} nicotine (△), and 4·5 mg kg^{-1} day^{-1} nicotine (▲). Cells were then incubated and assayed for corticosterone production. Values on the ordinate represent corticosterone production in the absence of ACTH.

(Lowry et al 1973). DNA content of stock cell suspensions was determined (Short et al 1968), and corticosterone production values were expressed as pmol μg^{-1} DNA. ED50 values were measured from dose-response curves determined by computer spline fit of the fluorogenic steroid production values.

Statistical analysis. The results are presented as the mean ± s.d. Each data point on the graphs represents 3 different incubations. All statistical comparisons between experimental and control groups were made using analysis of variance (ANOVA) followed by Newman-Keuls test. A value of $P < 0.05$ was considered to be statistically significant.

Results

Adrenal gland and cell morphology. The adrenal glands from different drug treatment groups showed no apparent differences in appearance or tissue weight. The mean adrenal weights were: 21·5 ± 2·1 g for the control group; 21·3 ± 1·6 g for the cocaine-treated group; 21·3 ± 1·5 g for the 1·5 mg kg^{-1} day^{-1} nicotine-treated group and 20·7 ± 1·0 g for the 4·5 mg kg^{-1} day^{-1} nicotine-treated group. Preparations of adrenal cell suspensions were examined by phase contrast microscopy, and no morphological differences could be detected among groups.

Adrenocortical cell steroidogenic response to ACTH stimulation. The sensitivity of adrenocortical cells to ACTH and the maximal steroidogenic capacity of cells were assessed by measuring the ACTH dose-steroidogenic response profile of cells obtained from the different treatment groups. The dose-response for the cell suspension prepared from the control group is shown in Fig. 1. The maximum rate of glucocorticoid production was approximately 700 pmol (μg DNA)$^{-1}$ h^{-1}. The ED50 value was 10 pM for ACTH.

Similar results were obtained with cell suspensions prepared from rats implanted with osmotic minipumps and rats without pump implantation. Cell suspensions derived from rats treated with cocaine displayed the same maximum steroidogenic value as control rats. However, adrenocortical cells from cocaine-treated rats were approximately twice as sensitive to stimulation by ACTH (ED50 5 pM) as control rats. Non-stimulated
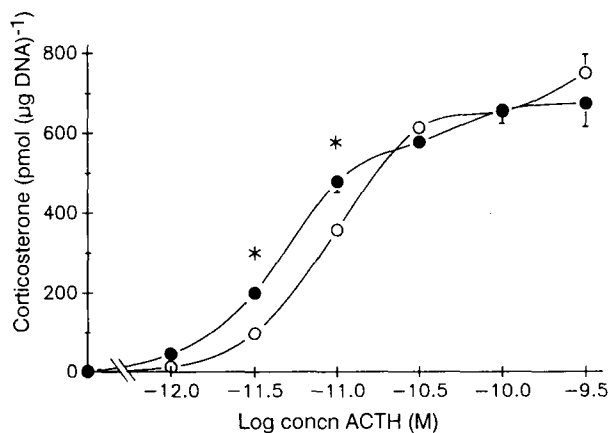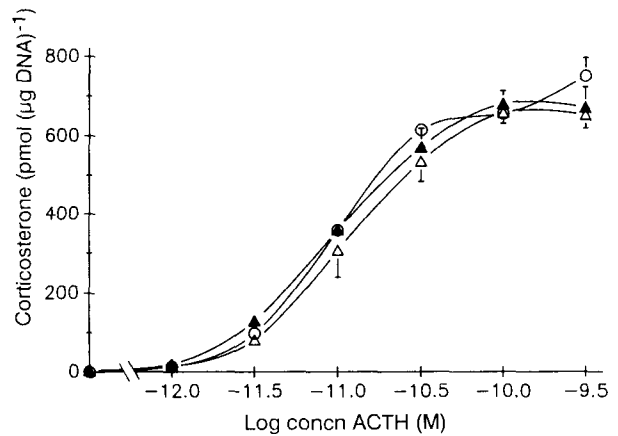

FIG. 1. Chronic cocaine treatment increases adrenocortical cell sensitivity to ACTH. Adrenocortical cell suspensions were prepared from rats implanted with osmotic mini-pumps delivering saline (O) or 30 mg kg^{-1} day^{-1} cocaine in saline (●) as described in Materials and methods. Samples of 10000 cells in 0·48 mL were transferred to incubation tubes. ACTH was then added in a volume of 0·02 mL to the final concentration indicated on the abscissa. Cell suspensions were incubated for 60 min at 37°C and analysed for corticosterone production by fluorometric assay. The symbols represent the means of triplicate incubations; the error bars show the standard deviations. For ACTH concentrations marked by *, the control and cocaine cell suspension corticosterone production values are significantly different ($P < 0.05$). Values on the ordinate represent corticosterone production in the absence of ACTH.

corticosterone production values for cell suspensions derived from control and cocaine treated rats were both near zero (Fig. 1). Similar results were obtained when the data were expressed in terms of corticosterone production per 10^4 cells.

The results shown in Fig. 2 indicate that neither the low nor the high dose of nicotine significantly altered the maximum rate of corticosterone production or the sensitivity of cells to ACTH relative to control values.

The influence of bt$_2$cAMP and 20(R),22(R)-dihydroxy-cholesterol on stimulation of steroidogenesis by adrenocortical cell suspensions prepared from the different treatment groups was also examined to determine if post-adenylate cyclase regulatory steps or steroid hormone biosynthetic enzyme levels, respectively, were altered by drug treatment. Maximally stimulating levels of each of these compounds gave similar steroidogenic rates for cells derived from each treatment group (data not shown).

Effect of acute in-vitro cocaine or nicotine treatment on steroidogenesis in suspensions of adrenocortical cells. When nicotine or cocaine was added to adrenocortical cells at concentrations ranging from 0·1 to 100 μM, no increase in basal corticosterone production was seen (data not shown). Cell samples treated with 10 pM ACTH had a steroidogenic rate of 42 pmol corticosterone per 8000 cells per h, which was 60% of the maximally stimulated rate. Cocaine or nicotine was added (100 μM) to samples together with ACTH (10 pM). The steroidogenic rate observed with the cocaine plus ACTH treatment was essentially identical to the treatment with 10 pM ACTH alone. Adrenocortical cells incubated with 0·1 mM nicotine and 10 pM ACTH produced slightly more (12%) corticosterone than cells receiving just 10 pM ACTH. However, this difference was not statistically significant ($P > 0.05$).

Discussion

In this study the doses of nicotine produced plasma levels in rats similar to those found in man after 20-60 cigarettes a day had been smoked (Murrin et al 1987; Fung & Lau 1988). The dose of cocaine used (30 mg kg^{-1} day^{-1}) relates to concentrations found in drug users (Gregler & Marks 1986). Neither the technique nor the drugs used caused any overt sign of tissue infection or necrosis. We observed no changes in adrenocortical cell steroidogenesis in response to the nicotine treatments, but the cocaine treatment increased the sensitivity of cells to stimulation by ACTH.

No difference in adrenal weight was observed in any of the rats, in contrast to the finding of Balfour & Morrison (1975) who after giving daily subcutaneous injections of nicotine, recorded significant increases in adrenal weight. The difference may be due to the difference in duration and the mode of drug administration.

Cocaine treatment increased the sensitivity of cells to stimulation of steroidogenesis by ACTH approximately two-fold. Since our measurements were made with isolated, purified adrenocortical cells, this effect appears to be a direct, relatively stable alteration of adrenocortical cells. This assertion is supported by the finding that treatment of adrenocortical cells prepared from naive rats did not show differences in steroidogenesis when cocaine was added to the incubation medium. In addition, this increased sensitivity is not the result of a cocaine-induced increase in the basal rate of corticosterone production, because non-stimulated corticosterone production values were essentially identical for cells derived from control and cocaine-treated rats (Fig. 1). It is possible that cocaine alters the efficiency of signal transmission between ACTH receptors and adenylate cyclase, which in turn leads to the observed change in ACTH

responsiveness. Ramachandran (1987) has suggested that differences at this point in the regulatory pathway may account for differences in ACTH sensitivity observed between rat and human adrenocortical cells, which have ACTH receptors with similar ACTH affinities. Consistent with this suggestion is our finding that steroidogenic responses to bt$_2$cAMP and 20(R),22(R)-dihydroxycholesterol were similar in cells prepared from saline- and cocaine-treated rats. These two compounds stimulate steroidogenesis at sites in the regulatory pathway after adenylate cyclase (Free et al 1971; Falke et al 1975).

Nicotine has previously been reported to be a steroidogenic agonist in suspensions of cat adrenocortical cells (Rubin & Warner 1975) in the 60–600 μM range. In contrast, we observed no stimulation of steroidogenesis in the 0·1–100 μM range. These differences may be due to species differences in adrenocortical cell sensitivity to nicotine. However, it seems unlikely that a direct agonist effect of nicotine would contribute significantly to the observed stimulation of steroidogenesis associated with cigarette smoking in man, unless human adrenocortical tissue concentrates nicotine effectively. For example, the lowest dose at which stimulatory effects of nicotine were observed with cat adrenocortical cell suspensions (60 μM, Rubin & Warner 1975) is more than two orders of magnitude higher than peak plasma nicotine levels commonly encountered after cigarette smoking (Seyler et al 1984).

Cocaine (Rivier & Vale 1987) and nicotine (Fuxe et al 1989) have been shown to cause substantial increases in ACTH secretion. Nicotine administration and cigarette smoking have also been shown to elevate glucocorticoid levels. Our finding that chronic nicotine treatment of rats has no influence on adrenocortical responsiveness to ACTH supports the view that nicotine influences glucocorticoid production by acting on the hypothalamus (Fuxe et al 1989) and not by direct actions on the adrenal cortex. Plasma corticosterone levels of rats exposed to nicotine by repeated intraperitoneal injection have previously been shown to become nicotine-insensitive after approximately four weeks of treatment (Cam & Basset 1983). Similarly, the nicotine-induced increase in plasma ACTH levels is smaller in rats that received previous nicotine injections (Sharp & Beyer 1986).

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