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Serotonin-3 Receptor and Ethanol-Stimulated Dopamine Release in the Nucleus Accumbens

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CAMPBELL, A. D. AND W. J. McBRIDE. *Serotonin-3 receptor and ethanol-stimulated dopamine release in the nucleus accumbens.* PHARMACOL BIOCHEM BEHAV 51(4) 835-842, 1995.—The present study was undertaken to examine the involvement of activation of 5-HT$_3$ receptors in the rat nucleus accumbens (Acb) on the effects of ethanol-induced increases of dopamine (DA) using the selective agonist 1-(m-chlorophenyl)-biguanide (CPBG). Perfusion of CPBG through the microdialysis probe concentration-dependently (3.3–100 μM) enhanced the extracellular levels of DA in the Acb. Extracellular DA concentrations increased as high as 1000% of baseline. The CPBG-induced increases in DA levels were Ca^{++} dependent and inhibited by local perfusion with the 5-HT$_3$ antagonist ICS 205-930 (100 μM). In addition, CPBG at high concentrations caused significant decreases in the extracellular levels of DA metabolites. Intraperitoneal (IP) injection of 1 g/kg ethanol produced no changes in extracellular DA levels in the Acb; coadministration of 1 g/kg ethanol (IP) and 5 μM CPBG (local) produced increases equal to 5 μM CPBG alone. Administration of 2 g/kg ethanol (IP) alone enhanced extracellular DA levels by ~60% above baseline, whereas local perfusion of 5 μM CPBG alone produced an increase of ~100% above baseline. The coadministration of 2 g/kg ethanol (IP) and 5 μM CPBG (local) enhanced DA levels by approximately 170% above baseline; this apparent additive enhancement was almost completely prevented when 100 μM ICS 205-930 was locally coperfused. Local administration of 3.3–100 μM CPBG did not alter the extracellular levels of serotonin or 5-hydroxyindoleacetic acid. The results support an involvement of 5-HT$_3$ receptors in regulating DA release in the Acb, and also in mediating ethanol-induced DA release. However, the in vivo interaction of ethanol and 5-HT at the 5-HT$_3$ receptor within the Acb is clearly not synergistic.

Nucleus accumbens Dopamine Serotonin 5-HT$_3$ receptor Ethanol Microdialysis
1-(m-chlorophenyl)-biguanide ICS 205-930

THE NUCLEUS accumbens (Acb) and, more generally, the mesolimbic dopamine (DA) system are thought to play a major role in the rewarding properties of drugs of abuse (20,32), as it appears that the ability of an agent to enhance extracellular DA levels in the Acb is a shared characteristic among drugs commonly abused (7,10,11). The presence of 5-HT$_3$ receptors in the mesolimbic DA system has led to speculation that these receptors may somehow modulate the activity of DA neurons and therefore may be involved in the rewarding properties of abused drugs. Recent evidence supports such a hypothesis. For example, 5-HT$_3$ antagonists have been shown to decrease the number of spontaneously active ventral tegmental area (VTA) DA neurons (25,30), block the reinforcing properties of morphine and nicotine (6), suppress voluntary ethanol intake (12,19), and block the discriminative stimulus properties of ethanol (14). In vivo studies using microdialysis have shown that 5-HT$_3$ antagonists inhibit the increase in extracellular DA levels in the Acb elicited by morphine (5,27), nicotine (5), cocaine (24), and ethanol (33,35). In addition, 5-HT$_3$ receptor agonists have been shown to mediate increases in extracellular DA concentrations both in vitro (3,4) and in vivo (1,9,15). However, studies with 2-methyl serotonin (a 5-HT$_3$ agonist) in the perfusate confound detection of endogenous DA, whereas results with 1-phenylbiguanide (another 5-HT$_3$ agonist) have suggested an action at the DA transporter (17,31). Therefore, one objective of the present study was to characterize the effects of the 5-HT$_3$ agonist, 1-(m-chlorophenyl)-biguanide (CPBG), on endogenous DA release in the Acb. This compound has been reported to be more selective than 1-phenylbiguanide (18) and, unlike 2-methyl serotonin, does not interfere with the HPLC analysis of endogenous extracellular DA levels.

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Increases in extracellular DA concentrations in the Acb observed following acute ethanol exposure have been well documented (10,16,35). Mechanistic studies in vivo have shown that the ethanol-enhanced increases in extracellular DA and 5-HT levels in response to pharmacologically relevant doses of ethanol are inhibited by $5\text{-}HT_3$ antagonists given either peripherally (5) or locally (33,35). Electrophysiologic evidence, using neuroblastoma cells and isolated neurons, also supports a selective effect of ethanol on the $5\text{-}HT_3$ receptor, as ethanol potentiates ion current through the receptor and enhances the response to agonist; both effects are blocked by the $5\text{-}HT_3$ antagonist ICS 205-930 (22). Taken together, these data support a role for the 5-HT system and, more specifically, the $5\text{-}HT_3$ receptor, in the actions of ethanol and other drugs of abuse. To date, however, no studies have been conducted to examine the interaction of ethanol with the activated $5\text{-}HT_3$ receptor in vivo. The present study was conducted to examine further the role of this receptor subtype in the ethanol-enhanced release of DA and/or 5-HT in the Acb using in vivo microdialysis to measure extracellular levels of the monoamines and their main metabolites.

METHOD

Surgery

Female Wistar rats (275-300 g) were implanted with microdialysis probes under halothane anesthesia. The loop-style probes were made essentially as described elsewhere (28,29), except that they were secured in 18-ga thin-wall stainless-steel tubing, which allowed for more accurate placement during surgery. The dialysis membrane was 2 mm in length (approximately 4 mm total exposed length) and the tip projected approximately 4 mm beyond the stainless-steel tubing to ensure no contact of the stainless-steel tubing with the Acb. Rats were placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA), their skulls were exposed, and a small hole was drilled to insert the microdialysis probe. Animals were maintained on a 37°C heating pad throughout the course of the surgery. Probes were implanted in the Acb according to the atlas of Paxinos and Watson (26) using the following coordinates relative to Bregma: AP + 1.4 mm, L + 2.4 mm, and D/V −8.4 mm. The probe was slowly lowered (1 mm/min) into position angled toward the midline at 10° from the vertical; two small stainless-steel screws were placed in the skull to secure the probe, and the probe was fixed with cranioplastic cement (Plastics One, Roanoke, VA). The animals were allowed 24 h to recover from surgery, during which time they were allowed free access to food and water.

Microdialysis

Experiments were performed in awake, freely moving animals in their home cage. A liquid swivel (BAS, W. Lafayette, IN) was used to connect the probe to the microinfusion pump. Artificial cerebrospinal fluid (aCSF) (composition in mM: NaCl, 145; KCl, 2.7; $MgCl_2$, 1.0; $CaCl_2$, 1.2; pH 7.4 with NaH_2PO_4) was filtered through a 0.2 μm sterile filter and perfused through the probe at 1.2 μl/min with a syringe pump (Harvard Instruments, South Natick, MA) for 60–90 min before baseline samples were collected. Baseline samples were collected every 20 min for 60 min or until a stable baseline of DA and 5-HT was reached (usually within 60 min). 1-(m-chlorophenyl)-biguanide (CPBG) (RBI, Natick, MA) or ICS 205-930 (Sandoz, East Hanover, NJ) dissolved in aCSF was perfused through the probe to determine their effect on the extracellular levels of DA and the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and 5-HT and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA). Ethanol was administered intraperitoneally (IP) in a 15% (v/v) solution with 0.9% sterile saline. Ca^{++} dependency was determined with aCSF containing no $CaCl_2$ (increase $MgCl_2$ to 2.2 mM to maintain osmolarity) and 1 mM EGTA. Dialysates were collected in vials containing 10 μl of 0.1 M HCl, and were either analyzed directly or immediately frozen on dry ice and stored at −70°C until analysis. Frozen samples showed no signs of degradation for up to 1 month. At the end of each experiment, a 1% solution of bromphenol blue in aCSF was perfused through the probe to verify placement in the Acb. The animals were then overdosed with halothane and decapitated, and the brains removed. Brains were stored at −70°C. Frozen sections were prepared and probe placement was verified according to the atlas of Paxinos and Watson (26). Ninety percent of the surgeries resulted in probe placement ending within the medial (shell) Acb; the remaining 10% ended more laterally within the core. However, even when probes appeared to end mainly in the core there was still overlap into the shell portion in most cases. Data obtained when placements were clearly not perfusing a portion of the shell (medial) were not used.

Dialysate Analysis

Samples were analysed by HPLC with electrochemical detection. The analytical column (Spherisorb 3 μm ODS2, 2 × 150 mm; Keystone Scientific, Bellefonte, PA) was connected to a Rheodyne 20-μl injection loop and Isco Model 2350 pump (Isco, Lincoln, NE). Mobile phase consisted of 75 mM sodium acetate, 65 mg/l sodium octanesulfonic acid, 0.5 mM EDTA (Sigma, St. Louis, MO), and 10% methanol; the pH was adjusted to 4.4 with acetic acid. The flow rate was 0.2 ml/min and the column was kept at 40°C. The electrochemical detector (EG&G PARC, Princeton, NJ) was equipped with a dual glassy carbon electrode to detect DA, 5-HT, and the metabolites DOPAC, 5-HIAA, and HVA. DA and 5-HT were assayed at a potential of 600 mV, and a sensitivity setting of 0.2 nA/V. The metabolites were assayed at a potential of 750 mV with a sensitivity setting of 20 nA/V. The outlet of both channels was sent to a Chrom Perfect (Justice Innovations, Inc., Palo Alto, CA) chromatography data analysis system to quantitate the amount of sample in 20 μl. The detection limit for DA and 5-HT was approximately 5 fmol injected onto the column.

Data Analysis

Because of subject-to-subject variability in the levels of extracellular monoamines and their metabolites, all data were normalized and expressed as percent of baseline. Normalized data were analysed by ANOVA to determine the significance of any drug effects on the extracellular levels of DA, 5-HT, DOPAC, HVA, or 5-HIAA. Percent of baseline levels for each treatment were determined by dividing the mean of the 40- and 60-min drug perfusion samples (maximum response) by the mean of three baseline samples within each subject × 100. Data were analysed by comparing the mean value of the 40- and 60-min drug perfusion samples (maximum response) to control values in which animals were perfused with aCSF alone, injected with saline, or compared to another treatment as described in the figure legends. Significance of multiple comparisons were determined by ANOVA followed by the Dunnett's post hoc test. The significance level was set, a priori,

at $p < 0.05$. All values presented are uncorrected for probe recovery efficiency, which has been shown previously to be between 15 and 20% for all compounds of interest (28).

RESULTS

The time course of 33 μM CPBG-stimulated DA release indicated that the maximum response occurred after 40–60 min (Fig. 1A). DA levels had returned to control levels within 60 min of terminating the drug perfusion. Other concentrations (3.3, 5, 10, 33, 50, and 100 μM) of CPBG showed similar effects on the time course of DA release (data not shown). In contrast, the extracellular levels of the DA metabolites DOPAC and HVA were reduced by the same treatment and did not return to baseline even after 3 h following perfusion with 33 μM CPBG (Fig. 1B).

Using the values from the 40- and 60-min time periods, it can be seen that CPBG concentration-dependently enhanced the extracellular concentrations of DA, but not 5-HT, in the Acb (Fig. 2A). Because the sensitivity of the electrochemical detector was set at a high level (0.2 nA/V) to detect any possible changes in 5-HT levels, accurate quantitation of DA levels at higher concentrations of CPBG was difficult (because of current overload and subsequent peak cutoff), as can be observed by the large amount of variability in the data at the two highest concentrations (50 and 100 μM). In addition, preliminary experiments showed that another 5-HT_3 agonist, 2-methyl-5-HT, interefered with the elctrochemical detection of 5-HT, and no further experiments were performed with this compound.

Changes in the extracellular levels of the major metabolites of DA and 5-HT following perfusion of various concentrations of CPBG can be seen in Fig. 2B. Decreases in the levels of DOPAC and HVA occured at the highest concentrations of CPBG (33–100 μM) but not at the lower concentrations (3.3–10 μM) that significantly enhanced extracellular DA levels. No changes in the extracellular levels of 5-HIAA were observed at any dose, which is consistent with the lack of effect of CPBG on extracellular 5-HT levels.

The Ca^{++} dependency of basal and evoked increases in extracellular DA concentrations in the Acb using the described microdialysis method was investigated. Basal release of DA was approximately 50% Ca^{++} dependent in our system, as determined by perfusing aCSF without Ca^{++} and containing 1 mM EGTA (Fig. 3). The response to 10 μM CPBG was also 50% Ca^{++} dependent, suggesting a 5-HT_3 receptor-mediated response to 10 μM CPBG using our methodology. Also, 100 μM ICS 205-930, a 5-HT_3 receptor antagonist, blocked the effects of 10 μM CPBG on extracellular DA concentrations without its causing any changes in basal DA concentrations (Fig. 4). Even after removal of ICS 205-930 from the perfusion medium, DA release remained sensitive to CPBG (Fig. 4).

To investigate a possible synergistic interaction between ethanol and the 5-HT_3 receptor, the effects of different combinations of the two compounds on extracellular DA levels were studied. IP administration of 1 g/kg ethanol caused no observed changes in extracellular DA concentrations in the Acb (Fig. 5). Perfusion of 5 μM CPBG in combination with 1 g/kg ethanol did not enhance DA release further than 5 μM CPBG alone (Fig. 5). No changes in the extracellular levels of DOPAC or HVA were observed following IP administration of 1 g/kg ethanol with or without local perfusion of 5 μM CPBG (data not shown). In addition, the effects of 3.3 μM CPBG (which caused a small increase in DA release that was not statistically significant) in combination with 2.0 g/kg ethanol (IP) were studied (Fig. 6). The 2.0-g/kg dose of ethanol caused a significant increase in the extracellular levels of DA of ~60% above control values; the combination of ethanol

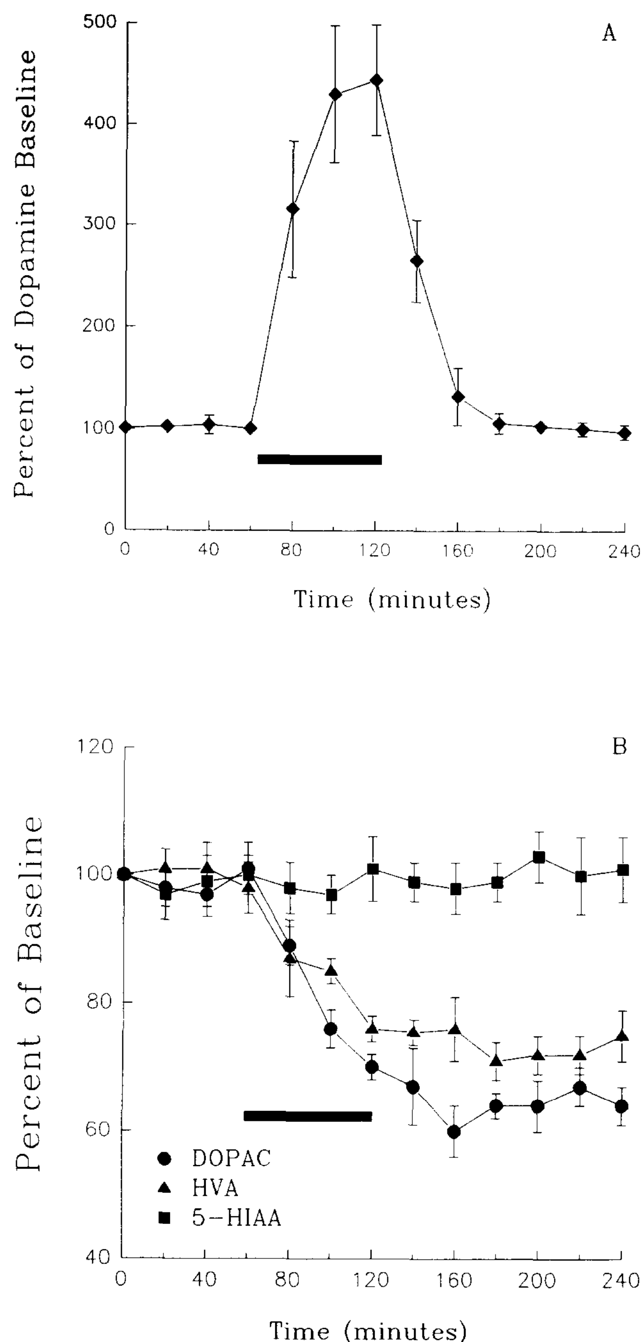

FIG. 1. Time course of changes in extracellular levels of DA (A) and the monoamine metabolites DOPAC, HVA, and 5-HIAA (B) following perfusion of 33 μM CPBG for 60 min. After establishment of a stable baseline, CPBG in aCSF was perfused for 60 min through the microdialysis probe (bar) and the perfusate was then changed to aCSF alone. Perfusion continued for another 2 h following removal of CPBG. The levels of metabolites did not return to basal for at least 3 h (longest time point collected; not shown). Results represent mean ± SEM of five to seven experiments.

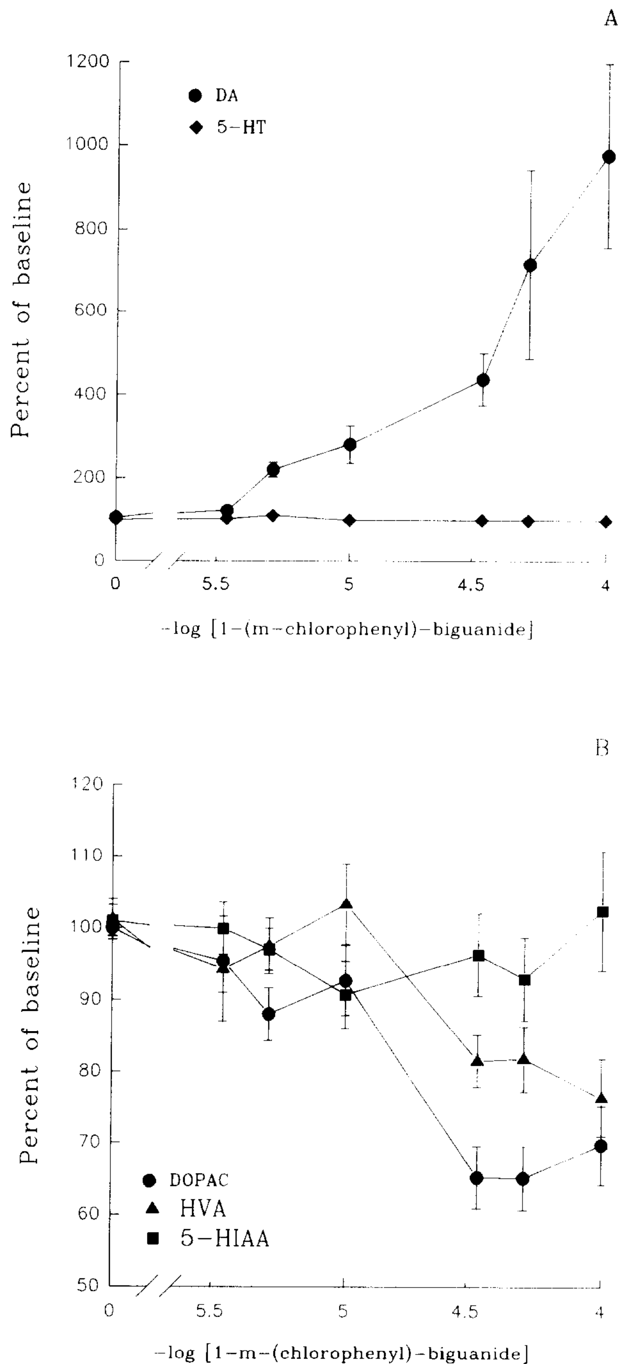

FIG. 2. Concentration-response effects for CPBG-induced changes in the extracellular levels of DA, 5-HT (A) and their main metabolites (B) in the Acb. CPBG (3.3, 5, 10, 33, 50, and 100 μM) was perfused through the microdialysis probe as described in Method. Each animal received only one concentration of drug. After establishment of a stable baseline, CPBG was perfused for 60 min through the probe, and the data from the 40- and 60-min samples were compared to baseline. ANOVA followed by a Dunnett's post hoc test revealed a significant dose effect for DA [$F(6, 45) = 7$, $p < 0.0001$], DOPAC [$F(6, 54) = 11.3$, $p < 0.0001$], and HVA [$F(6, 55) = 4.5$, $p < 0.001$], but not 5-HT [$F(6, 33) = 0.1$] or 5-HIAA [$F(6, 45) = 1.4$]. Each point represents the mean ± SEM of six to 12 experiments. Basal levels/20 min sample were as follows: DA, 32 ± 4 fmol (n = 46); 5-HT, 20 ± 2 fmol (n = 40); DOPAC, 11 ± 1 pmol (n = 55); 5-HIAA, 3 ± 0.3 pmol (n = 52); and HVA, 5 ± 0.3 pmol (n = 56).

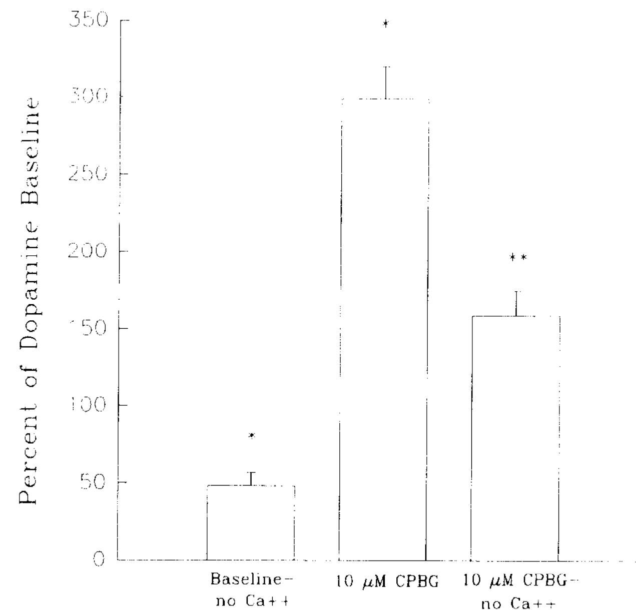

FIG. 3. Ca^{++} dependency of DA release in the Acb. After establishment of a stable baseline, aCSF was switched to one containing no Ca^{++} and 1 mM EGTA. Then, 10 μM CPBG was perfused in regular aCSF or aCSF containing no Ca^{++} and 1 mM EGTA. Each animal received only one treatment of CPBG, and the data were compared to baseline values by ANOVA. Data represent mean ± SEM of eight to nine experiments. *Significantly different from baseline value, $p < 0.01$. **Significantly different from 10 μM CPBG with Ca^{++}, $p < 0.01$.

with 3.3 μM CPBG produced an apparent additive effect (Fig. 6) that was not significantly different than the 2-g/kg treatment alone. The extracellular levels of 5-HT were slightly increased after injection of ethanol alone; the local perfusion with CPBG did not change this response (data not shown).

Local perfusion with 5 μM CPBG caused an increase in extracellular DA levels of 80–100% above baseline concentrations (Fig. 7); this response was blocked by coperfusion with 100 μM ICS 205-930. IP administration of 2.0 g/kg ethanol enhanced extracellular DA concentrations ~60% above baseline. The local perfusion with 5 μM CPBG and simultaneous injection of 2.0 g/kg ethanol caused an increase in extracellular DA concentrations of ~150% above baseline levels (Fig. 7). The response to 5 μM CPBG plus 2 g/kg ethanol was antagonized by local administration of 100 μM ICS 205-930. No changes in the extracellular levels of DOPAC or HVA were observed following IP administration of ethanol alone or in combination with local perfusion of 3.3 or 5 μM CPBG (data not shown).

DISCUSSION

The present results provide additional support for a role of $5\text{-}HT_3$ receptors in regulating DA release in the Acb. These in vivo findings are in agreement with studies in the Acb and other brain regions (1,8,9,15) where 1-phenylbiguanide or 2-methyl-5-HT was used as an agonist. However, no other studies have used the more selective CPBG to investigate the role of $5\text{-}HT_3$ receptors in modulating DA release in vivo. CPBG potently enhanced the extracellular concentrations of DA in a concentration-dependent manner. The effects were also re-

versible (levels returned to baseline within 40 min of termination of perfusion), partially Ca^{++} dependent, and sensitive to the actions of the 5-HT_3 antagonist ICS 205-930 (Figs. 1, 3, and 4). A complete Ca^{++}-dependent effect on CPBG-stimulated DA efflux was not observed because Ca^{++} is still present in the surrounding tissue. However, the 50% reduction in both basal and stimulated DA efflux would support receptor-regulated, exocytosis-mediated release of DA. In addition, 5-HT_3 receptors do not appear to have a tonic effect on DA release, as ICS 205-930 perfusion alone did not alter extracellular baseline levels of DA (Fig. 4). Furthermore, similar to the results obtained in the medial prefrontal cortex in vivo using the less selective 1-phenylbiguanide (8), CPBG-mediated changes in DA were of short duration (Fig. 1) and did not alter DOPAC or HVA extracellular levels at lower concentrations.

The specificity of phenylbiguanide as an agonist at the 5-HT_3 receptor has come under question recently as a result of its putative activity at the DA transporter (2,17,31). However, the addition of chloride to phenylbiguanide has made the compound more selective and potent at the 5-HT_3 receptor and less likely to interact with the DA transporter (18). The results of the present study support a receptor-specific effect of CPBG on DA release in the Acb at low to moderate concentrations. The reduced levels of DOPAC and HVA observed at the highest concentrations of CPBG (Fig. 2B) suggest inhibition of the DA transporter, as has been proposed for 1-phenylbiguanide (31). Indeed, the use of very high concentrations of 1-phenylbiguanide has been common in in vivo (8,9) and in vitro (2,3,31) studies. The effects of low μM concentrations of 1-phenylbiguanide or CPBG have not previously been investigated in vivo. Further studies investigating the Ca^{++} dependency and the antagonist inhibition of the DA-releasing effects seen at higher concentrations are necessary to determine the specificity of the effect at these concentrations. Regardless, CPBG is a potent stimulator of DA release, because the in vivo concentrations of CPBG are most certainly less than that perfused through the probe.

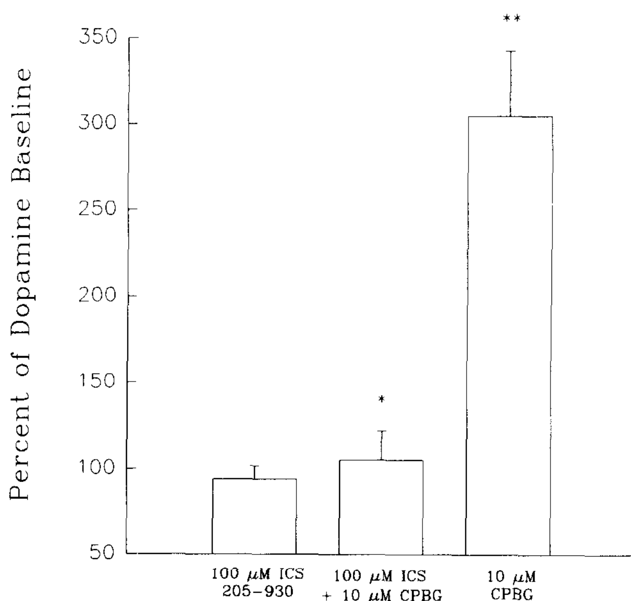

FIG. 4. Inhibition of 10 μM CPBG-induced DA release by ICS 205-930. The bar graph represents the order of perfusion through the probe. After establishment of a stable baseline, 100 μM ICS 205-930 was perfused through the microdialysis probe 60 min before the addition of 10 μM CPBG. The compounds were then coperfused for 60 min. The aCSF was then switched to one containing only CPBG, and samples were taken for 60 min. The 40- and 60-min samples were used in all comparisons, which were performed by ANOVA. Results represent mean ± SEM of six to 10 experiments per treatment. *Significantly different from perfusion with 10 μM CPBG alone, $p < 0.001$. **Significantly different from baseline, $p < 0.001$.

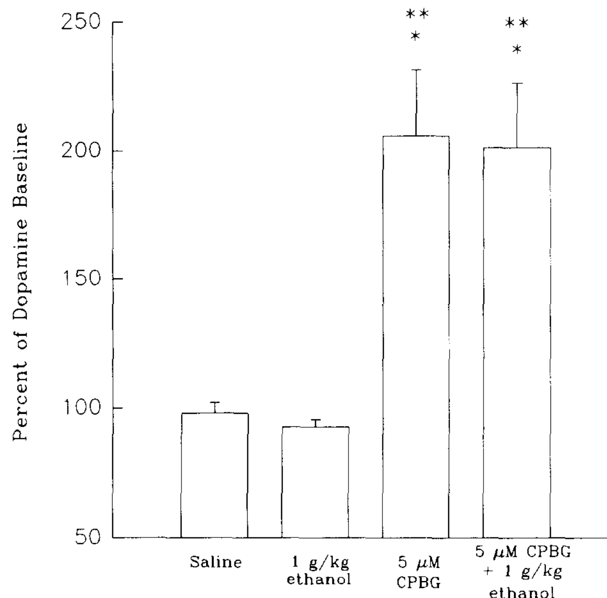

FIG. 5. The combined effects of IP administration of 1 g/kg ethanol and 5 μM CPBG on extracellular DA levels in the Acb. Animals were injected with saline, 1 g/kg ethanol, or perfused with 5 μM CPBG in the Acb alone and in combination with IP administration of 1 g/kg ethanol. All animals received only one treatment. The combination of 1 g/kg ethanol and 5 μM CPBG was performed simultaneously, and the average of the 40- and 60-min time points was used for all comparisons (ANOVA). Results represent mean ± SEM of six to 10 experiments per treatment. *Significantly different from saline injection, $p < 0.01$. **Significantly different from 1 g/kg ethanol, $p < 0.01$.

In agreement with other studies (5,35), IP administration of 2 g/kg ethanol significantly enhanced the extracellular concentrations of DA in the Acb (Figs. 6 and 7). In contrast to some reports (10,16), in the present study 1 g/kg had little effect on extracellular DA levels (Fig. 5). However, more recent studies (35,36) indicated that an IP dose of 1 g/kg ethanol produced a much smaller and less consistent increase in the extracellular concentrations of DA in the Acb. The reason for this difference is not known; however, several possibilities exist. First, and probably most important, the microdialysis procedures used were markedly different. In the former studies, there was undoubtedly more tissue damage (which could result in enhanced tissue sensitivity) caused by either the placement of two transverse probes (10,16) or by the placement of a concentric probe 4 h before initiation of the experiments (35,36). Second, probe placement in an anatomically heterogeneous brain region may also account for some of the difference. Third, the strains and/or sex of the rats used were different, which may have led to differences due to physiologic factors such as estrous cycles. Also, other studies used high Ca^{++} concentrations in the perfusate (10,16), which may en-

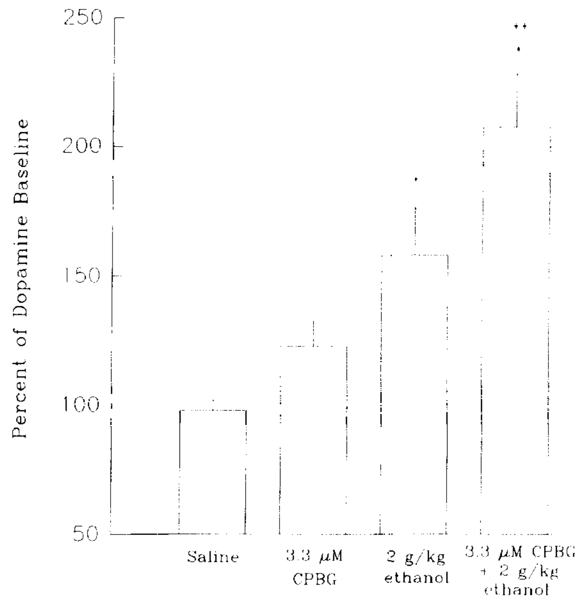

FIG. 6. The combined effects of IP administration of 2 g/kg ethanol and 3.3 μM CPBG on extracellular DA levels in the Acb. Animals were injected with saline, 2 g/kg ethanol, or perfused with 3.3 μM CPBG in the Acb alone and in combination with IP administration of 2 g/kg ethanol. All animals received only one treatment. The combination of 2 g/kg ethanol and 3.3 μM CPBG was performed simultaneously, and the average of the 40- and 60-min time points was used for all comparisons (ANOVA). Results represent mean ± SEM of six to eight experiments per treatment. *Significantly different from saline injection, $p < 0.005$. **Significantly different from 3.3 μM CPBG and saline, $p < 0.001$.

hance the effects of a pharmacologic manipulation. Although not shown, 2 g/kg ethanol enhanced the extracellular levels of 5-HT in the Acb, in agreement with previous observations (35). The increases observed presently were not significant, however, because of a great deal of variability in the data. The most obvious explanation for this discrepancy is the time between placement of probe and initiation of the experiment (4 h vs. 24 h in the present study). Other factors may include those described earlier.

Until now, evidence for a 5-HT_3 receptor involvement in the response to ethanol has come only from inhibiting this response with antagonists [(5,33,35); also see present study]. In an attempt to assess involvement of 5-HT_3 receptor activation in ethanol-stimulated DA release, various doses of both CPBG and ethanol in combination were examined (Figs. 5-7). The results from all combinations suggested an additive effect of the two compounds on stimulating DA release in the Acb. One possibility is that CPBG and ethanol are acting at independent sites. However, the observation that 100 μM ICS 205-930 was able to inhibit the response to the combination (Fig. 7) supports the idea that 5-HT_3 receptors are mediating both effects. The 100-μM concentration of ICS 205-930 perfused through the dialysis probe is similar to other reports (35) and is considerably less than the concentration on the tissue side of the dialysis membrane (34). However, because the concentration on the tissue side is unknown, a nonspecific effect by ICS 205-930 cannot be ruled out. Furthermore, the present finding of an additive action of CPBG and ethanol on DA efflux is in agreement with electrophysiologic data (22), where potentiation (not synergism) by ethanol of the agonist-induced ion flux was observed in isolated neurons.

Activation of 5-HT_3 receptors in the Acb did not alter extracellular levels of 5-HT or 5-HIAA (Fig. 2). These results suggest that 5-HT_3 receptors are not located presynaptically on 5-HT nerve terminals to regulate 5-HT release. However, in the present study, the basal levels of 5-HT are at the limit of detection of the assay, and it is impossible to determine whether any autoreceptor negative feedback was being regulated by 5-HT_3 receptors. The results of the present study, however, showed that 5-HIAA levels were not changed by any concentration of CPBG, further suggesting that 5-HT_3 receptors do not modulate 5-HT release in the Acb. On the other hand, activation of 5-HT_3 receptors by 2-methyl-5-HT increases the in vivo extracellular levels of 5-HT in the hippocampus (23). Whether this effect is a result of a nonspecific action of 2-methyl-5-HT or via an indirect mechanism involving 5-HT_3 receptors is not known.

It is not presently known whether 5-HT_3 receptors are directly on DA nerve terminals or are on other neuronal processes that in turn act upon DA nerve terminals, as this has not yet been directly investigated. Autoradiographic studies indicate low densities of 5-HT_3 receptors in the Acb and stria-

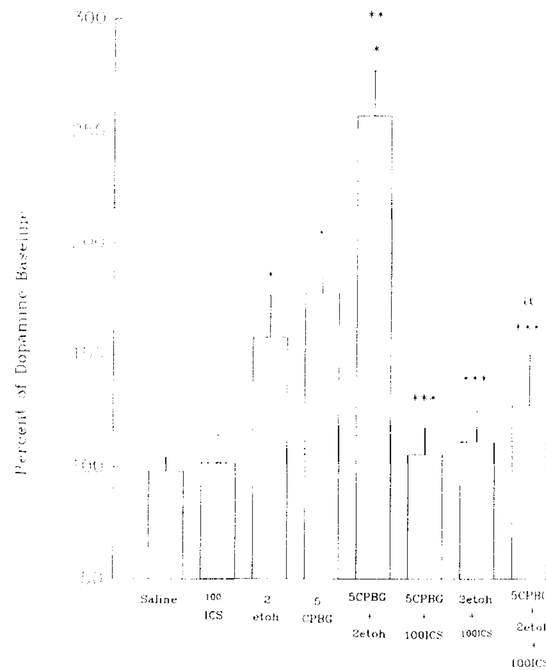

FIG. 7. Antagonism of the effects of 2 g/kg ethanol (2 etoh) alone or in combination with 5 μM CPBG (5 CPBG) by 100 μM ICS 205-930 (100 ICS) on extracellular DA release in the Acb. Animals were injected with saline, 2 g/kg ethanol, or perfused with 5 μM CPBG in the Acb alone or in combination with 100 μM ICS 205-930 and/or IP administration of 2 g/kg ethanol. All animals received only one treatment. The combination of 2 g/kg ethanol and 5 μM CPBG (with and without ICS 205-930) was performed simultaneously, and the average of the 40- and 60-min time points was used for all comparisons (ANOVA). *Significantly different from saline or baseline, $p < 0.01$. **Significantly different from 5 μM CPBG (alone) and 2 g/kg ethanol (alone), $p < 0.0005$. ***Significantly different from 5 μM CPBG (alone) or 2 g/kg ethanol (alone), $p < 0.05$. [a]Significantly different from 5 μM CPBG + 2 g/kg ethanol, $p < 0.0001$.

tum (13,21), two regions where there is evidence for 5-HT_3 receptor regulation of DA release (Figs. 1–4) (1). It is difficult to reconcile the disparity between low receptor density and potent modulation of DA release by CPBG. It is possible that through an as-yet unknown system, CPBG (or 5-HT_3 receptors) is able to amplify a process involved in DA release. Also, optimal parameters for in vitro binding assays for 5-HT_3 ligands may not be available, resulting in misleading data.

The data presented here support a specific role for 5-HT_3 receptors in mediating DA release in the Acb in vivo, and also support the involvement of 5-HT_3 receptors in ethanol-enhanced DA release in this region. Because the DA projections to the Acb have been implicated in the actions of drugs of abuse, and many drugs of abuse have been shown to preferentially enhance DA release in this region, further studies regarding the role of the 5-HT_3 receptor are warranted.

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