presence of any type of surfactant (Table 1). This suggests that cefazolin is not sufficiently bound to the micelles, because of its lower hydrophobicity (Yamana, Tsuji & others, 1977), to prevent or accelerate the degradation.

The interaction between anionic forms of penicillins, with lower lipophilicity (Tsuji, Kubo & others, 1977) and non-ionic micelles was significantly decreased. The K/N value at pH 6·5 for propicillin in POE (10 mM) micellar solution was found, by the dynamic dialysis method (Meyer & Guttman, 1968), to be 42 M^{-1}, indicating that about 70% of the penicillin anions exist in the free form.

A use for the non-toxic and non-ionic surfactant POE to improve oral bioavailability of acid-labile and poorly absorbed β-lactam antibiotics is proposed because there is: (1) an enhancement of gastro-intestinal absorption (Davis, Pfeiffer & Quay, 1970), (2) a stabilizing ability towards acid degradation, and (3) a good release of the substrate from the micelle at intestinal pH.

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Increased binding of [^{3}H]apomorphine in caudate membranes after dopamine pretreatment *in vitro*

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Most patients with Parkinson's disease being treated with L-dopa develop an increased frequency of abnormal involuntary movements during therapy (Barbeau, 1976). This progressively deteriorating response may be attributed to either the natural pathology of the disease (Meunter, Sharpless & others, 1977) or to an L-dopa-induced process of unknown origin (Martres, Costentin & others, 1977).

Although long-term exposure of β-adrenergic catecholamines generally results in tachyphylaxis or desensitization (Makman, 1971; Kebabian, Zatz & others, 1975; Franklin & Twose, 1976; Mukherjee & Lefkowitz, 1977), long-term administration of dopamine-mimetic drugs to animals sometimes produces 'behavioural facilitation' (Segal & Mandell, 1974; Friedman, Rotrosen & others, 1975; Klawans & Margolin, 1975; Short & Shuster, 1976; Kilbey & Ellinwood, 1977; but see the work of Martres & others, 1977 on single doses of apomorphine).

To investigate one possible molecular mechanism of this facilitation or sensitization induced by dopamine-mimetic drugs, we tested the effects of prolonged exposure (*in vitro*) of dopamine on the dopamine/neuroleptic receptors in the caudate nucleus of the calf.

Preparation of calf caudate homogenates. The experiments were done on crude homogenates of calf caudate, prepared as described by Seeman, Lee & others (1976, a,b). The homogenized tissues were finally resuspended in 10 vol of ice cold buffer (15 mM tris-HCl, pH 7·4, 5 mM Na_2EDTA, 1·1 mM ascorbic acid and 12·5 μM nialamide), incubated at 37° for 60 min, and stored frozen at −20° for several days or weeks until used further.

Pretreatment procedure. Before testing for the binding of [^{3}H]apomorphine, [^{3}H]haloperidol, ^{3}H-WB-4101 [^{3}H]2(*N*[2,6-dimethoxyphenoxyethyl])amino-methyl-1,-

* Correspondence.

4-benzodioxane) or [^{3}H]naloxone, the tissues were generally pretreated with dopamine or other drugs, as follows. The frozen homogenates were thawed, pooled and centrifuged at 44 000 *g* for 15 min at 4°. The supernatant was discarded and the pellets were resuspended in 5 ml buffer for every 300 mg of homogenate tissue. The suspension was subdivided into aliquots of 2 ml for the pretreatment step. Each 2 ml aliquot was incubated for 60 min (generally) at 37° in the presence of varying concentrations of dopamine and other compounds. Control samples were incubated for 60 min with buffer only. After 60 min pretreatment, the samples were flooded wth 30 ml buffer and rapidly centrifuged by simply turning off the (Sorvall RC-5) centrifuge switch after 31 000 *g* had been obtained in 60 s; a further 90 s were needed to bring the rotor to a halt. The flooding and centrifugation were repeated twice. The final pellet, resuspended in 4 ml of buffer, was then homogenized further by a Polytron homogenizer (Brinkmann Instrument Co.) at a setting of 7 (full scale =10) for 20 s, using a PT-10 generator (Polytron). The suspensions were then tested for binding of the ^{3}H-ligand.

General procedure for ^{3}H-ligand binding assays. The specific binding of the various ^{3}H-ligands was done by the general method previously described (Seeman & others, 1976a,b) with minor variations. The assays were done in glass test-tubes (12 × 75 mm) into which the following aliquots were placed: 0·2 ml buffer (with or without an excess of appropriate drug used to determine non-specific binding), 0·2 ml of the ligand and 0·2 ml of the homogenate suspension. After incubation at room temperature (20–21°) for 30 min, 0·5 ml of the mixture was filtered through Whatman glass fiber (GF/B) filters using a vacuum pump. The filters were washed with varying volumes of buffer (see later) and

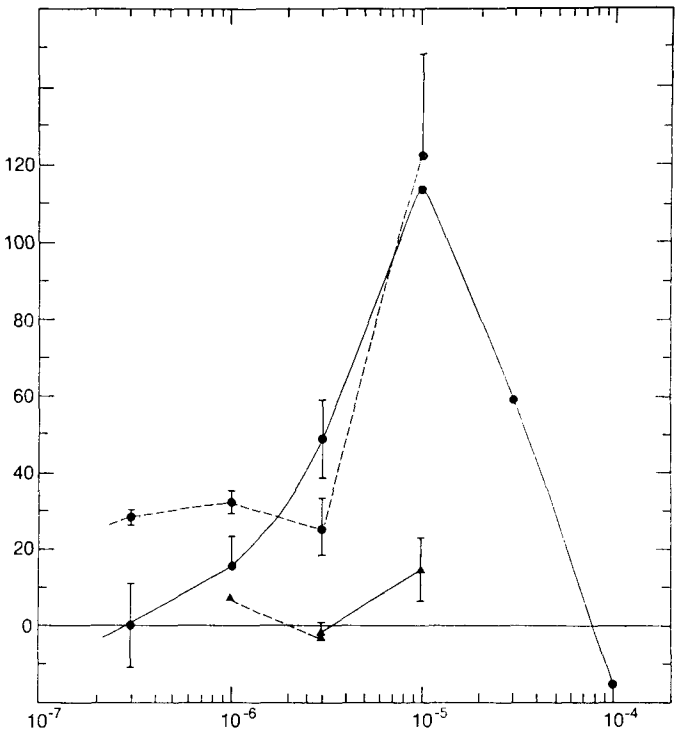

FIG. 1. The specific binding of [^{3}H]apomorphine (displaceable by (+)-butaclamol) increased after the *in vitro* exposure of the caudate homogenate to dopamine concentrations between 10^{-6} and 10^{-5} M. Higher pretreating concentrations of dopamine resulted in enough residual dopamine (after washing) to interfere with [^{3}H]apomorphine, thus accounting for the net inhibition of binding above 10^{-5} M dopamine. Pretreatment with (—)-noradrenaline also increased the specific binding over the concentration range, except that higher concentrations of (—)-noradrenaline did not interfere with the specific binding of [^{3}H]apomorphine. Clonidine and phenylephrine did not significantly alter [^{3}H]apomorphine binding. Vertical bars are s.e.m. for 3 to 15 experiments per point. Points without bars only represent two experiments. Control specific binding was approximately 30 fmol mg^{-1} protein, corresponding to about 150–200 specific c min^{-1} per assay tube and about 50% of total [^{3}H]apomorphine binding. Non-specific binding of [^{3}H]-apomorphine was not significantly affected. ●—● Dopamine; ●- -● noradrenaline; ▲—▲ clonidine; ▲- -▲ phenylephrine. Ordinate: % increase in specific [^{3}H]apomorphine binding. Abscissa: Drug concentration (M) in pretreatment.

then placed, without drying them, into liquid scintillation vials; 8 ml of Aquasol (New England Nuclear Corp., Boston) was added. The vials were stored at 4° for at least 6 h to permit the filters to become translucent. Each assay was done in quintuplicate.

Specific binding of [^{3}*H*]*apomorphine* (Seeman & others, 1976b). The specific binding was defined as that amount bound in the presence of 1·5 or 3 nM [^{3}H]apomorphine (14·1 Ci mmol^{-1}; custom-prepared for this laboratory in 1976 by New England Nuclear Corp., Boston) minus that amount bound in the presence of an excess of (+)-butaclamol (1 μM) or of apomorphine (200 nM). The filters using 1·5 nM[^{3}H]apomorphine were washed with 5 ml buffer, while those using 3 nM were washed with 14 ml buffer.

Specific binding of [^{3}*H*]*haloperidol* (Seeman, Chau-Wong & others, 1975; Burt, Creese & Snyder, 1976). The specific binding was defined as that amount bound in the presence of 2·5 nM [^{3}H]haloperidol (8·5 Ci mmol^{-1} at time of use, but originally 9·6 Ci mmol^{-1} when custom-synthesized for this laboratory by I. R. E. Belgique, Belgium, in 1974) minus that amount bound in the presence of an excess of (+)-butaclamol (100 nM). The filters were washed quickly (between 3 and 4 s) with 5 ml buffer.

Specific binding of [^{3}*H*]*naloxone* (Pert & Snyder, 1973). The specific binding was defined as that amount bound in the presence of 1 nM[^{3}H]naloxone (16·4 Ci mmol^{-1}; New England Nuclear Corp., Boston) minus that amount bound in the presence of an excess of morphine (500 nM). The filters were washed with 10 ml buffer.

Specific binding of ^{3}*H-WB-4101* (Greenberg, U'Prichard & Snyder, 1976). The specific binding was defined as that amount bound in the presence of 0·8 nM ^{3}H-WB-4101 (13·0 Ci mmol^{-1}; New England Nuclear Corp., Boston) minus that amount bound in the presence of an excess of reversible α-adrenoceptor blocking drug, phentolamine (3 μM). The filters were washed with 10 ml buffer.

The results in Fig. 1 show that the specific binding of [^{3}H]apomorphine increased after the calf caudate homogenate had been pretreated for 1 h with 3 μM dopamine. Similar treatment caused an increase in specific binding of [^{3}H]haloperidol (by 66 fmol mg^{-1}), but a decrease in [^{3}H]naloxone specific binding (by only 9 fmol mg^{-1}) and a small, not statistically significant, increase in ^{3}H-WB-4101 binding (see Fig. 2).

To examine the extent to which the dopamine had been washed out after the pretreatment period, a small amount of [^{3}H]dopamine was added to the membrane suspension during the pretreatment period and after extensive washing, sufficient remained to interfere with the subsequent binding of [^{3}H]apomorphine only if the pretreating concentration had been between 10^{-5} and 10^{-4} M (see Fig. 1).

Pretreatment with noradrenaline resulted in a similar pattern of enhanced specific binding of [^{3}H]apomorphine (Fig. 1). Any residual noradrenaline did not apparently

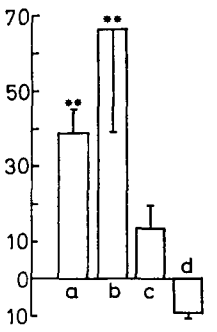

FIG. 2. The specific binding of [^{3}H]apomorphine (displaceable by 200 mM apomorphine) and [^{3}H]haloperidol to calf caudate tissue became increased after the homogenate had been pretreated for 1 h with 3 μM dopamine and washed extensively to remove all the dopamine. Similar treatment caused a less pronounced increase in ^{3}H-WB-4101 binding and a decrease in [^{3}H]naloxone binding. Values shown are means ±s.e.m. The 2 asterisks indicate statistical significance, at the 0·02 level, for at least 11 experiments. Each binding assay was done in quintuplicate. The absolute control value for specific [^{3}H]apomorphine binding was 29·2 fmol mg^{-1} protein, that for [^{3}H]haloperidol binding was 157·5 fmol mg^{-1}, while that for ^{3}H-WB-4101 was 150 fmol mg^{-1} and that for [^{3}H]naloxone was 75 fmol mg^{-1}. Pretreatment with agents other than dopamine was not tested with these radioligands. a: [^{3}H]Apomorphine; b: [^{3}H]haloperidol; c: ^{3}H-WB-4101; d: [^{3}H]naloxone. Ordinate: Change in specific binding (fmol mg^{-1}).

interfere with [^{3}H]apomorphine binding at the concentration remaining in the homogenate after washing. 1–3 μM phenylephrine, clonidine, phentolamine, isoprenaline and 5,6-dihydroxytryptamine did not elicit any significant increments in [^{3}H]apomorphine binding.

A separate series of experiments indicated that it took from 30–60 min of pretreatment with dopamine to elicit the enhanced specific binding of [^{3}H]apomorphine. Finally, it should be mentioned that pretreatment with haloperidol blocked the dopamine-induced effect, but the amount of haloperidol remaining (after the extensive washing) was always high enough to interfere with the subsequent binding of [^{3}H]apomorphine or [^{3}H]haloperidol; this apparent blocking action of haloperidol, therefore, was questionable.

The results indicate that pre-exposure of caudate homogenate with dopamine or noradrenaline *in vitro* leads to an increased binding of [^{3}H]apomorphine. This finding is rather surprising, considering that *in vitro* pre-exposure of various tissues with β-adrenergic catecholamines invariably leads to desensitization and *less* specific binding of the β-receptor radioligands, (−)-[^{3}H]alprenolol (Kebabian & others, 1975), (−)-[^{3}H]dihydroalprenolol (Mukherjee & Lefkowitz, 1977) or (+)-[^{3}H]propranolol (Nahorski, 1977). The only

report of tolerance or acute desensitization to a dopaminemimetic drug is that of Walters, Bunney & Roth (1975) who found that piribedil or apomorphine (i.p. or i.v.) desensitized dopaminergic cells in the rat substantia nigra.

Since the dopamine pretreatment did not produce statistically significant increments in the specific binding of the α-adrenoceptor ligand (^{3}H-WB-4101) or the opiate ligand ([^{3}H]naloxone), the effect on the [^{3}H]-apomorphine binding may be reasonably selective for dopamine receptors. However, the fact that both noradrenaline and dopamine were almost equipotent in producing this increase indicates that the effect was not extremely chemically specific. It is doubtful that the effect was simply an action of the catechol moiety since isoprenaline did not elicit any increase in the specific binding of [^{3}H]apomorphine.

There is no simple explanation for the present findings. Since the effective dopamine concentrations in the pretreatment medium were in the μM range and since dopamine-sensitive adenylate cyclase is also responsive to dopamine in the μM concentration range, the effect may have something to do with the generation of cyclic AMP *in vitro*. A second possibility is that during the pretreatment period the high concentration of dopamine may be converting or reconforming the receptors from the antagonist state to the agonist state (Creese, Burt & Snyder, 1975; Burt & others, 1976). This seems unlikely since the binding of both [^{3}H]-apomorphine and [^{3}H]haloperidol increased.

It is questionable whether the present findings have any significance for the behavioural sensitization or facilitation by dopamine-mimetic drugs, although this is possible. The behavioural effects usually take days or weeks to develop, while the *in vitro* effect reported in the present study occurs within ½ to 1 h.

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