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HYDROLYSIS OF DIESTER PRODRUGS OF APOMORPHINE*

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Abstract—*O,O'*-diesters of apomorphine exert behavioral effects identical to those of apomorphine, but prolonged in proportion to the bulk of the esters. This prolonged activity may reflect depot properties of the esters, and/or decreasing rates of hydrolysis to the presumably active metabolite, apomorphine. To test the latter hypothesis, mouse brain apomorphine was assayed by a fluorimetric method that can distinguish apomorphine from its monophenolic analogues and was confirmed by thin-layer chromatography. Apomorphine fluorescence was recovered after systemic injection of di-isobutyrylapomorphine as well as after apomorphine, but its half-life in brain was greatly prolonged after administration of the ester. Ester hydrolysis also occurred *in vitro* with minces or homogenates of rat tissue, yielding a product with the fluorescent and chromatographic properties of free apomorphine which also stimulated the activity of a dopamine-sensitive adenylate cyclase in rat striatal homogenates, sensitive to apomorphine but not its esters. Most of the activity resided in the high-speed supernatant fraction of liver; high esterase activity was found in rat plasma and was present in most tissues. This reaction was dependent on temperature, tissue and substrate concentration, followed saturable kinetics, and was inhibited by triorthocresylphosphate, an esterase inhibitor. This apparent enzymatic reaction proceeded at rates varying inversely with the size of the ester substituent. These results support the suggestion that the rate of hydrolysis may be related to desirable prolongation of action of ester prodrugs of apomorphine.

The administration of their labile, lipophilic precursors can facilitate the delivery of ordinarily highly hydrophilic pharmacologically active catecholamine agonists to the central nervous system (CNS). Examples of such "prodrugs" include *O*-triacetylated norepinephrine [1], the methylene-dioxyether trivastal (ET-495, Piribedil, converted *in vivo* to a catechol, S-584) [2], *O,O'*-diacetylapomorphine [3,4], and *O,O'*-diacetyl-*N,N*-dimethyldopamine [4,5]. Some of these compounds with CNS activity that are precursors or prodrugs of dopamine agonists might have clinically useful properties, for example as antiparkinson agents [4–6], and as antagonists of prolactin release [7–9]. Some prodrugs of this type might provide advantages in addition to improved availability to the CNS, including prolonged action.

Short activity has severely limited the clinical usefulness of apomorphine as a presumed dopamine agonist in the past [6]. In previous work, we found that a series of apomorphine esters produced behavioral effects in the rat similar to those of apomorphine itself, but with a prolonged duration of action [4,9,10]. Thus, a series of *O,O'*-diesters of apomorphine of increasing size provoked stereotyped gnawing behavior in intact rats, as well as rotation in a direction contralateral to unilateral midbrain lesions in one nigrostriatal pathway. The esters had a delayed latency to maximal behavioral effect as well as a prolonged duration of action in comparison with apomorphine; prolongation of the behavioral effects of the esters increased with the size of the ester substituent, up to six times that of apomorphine. None of the esters increased the synthesis of cyclic AMP in striatal homogenates exhibiting adenylate cyclase activity stimulated by apomorphine or dopamine.

These observations suggested that desirable prolongation of action of apomorphine by esteric derivatives is probably due to their increased lipophilic "depot" properties or to slow hydrolysis of the larger esters that converts the prodrugs to the probable active product, apomorphine. We now report that such esters can be hydrolyzed *in vitro* and *in vivo* to free apomorphine, evidently by an enzymatic process, the rate of which in inversely related to the size of the ester substituent, suggesting that the increased duration of action of these prodrugs may in part reflect the rate of production of an active metabolite, presumably apomorphine.

MATERIALS AND METHODS

Apomorphine·HCl was obtained from Merck. Diisobutyryl, dipropionyl, dipivaloyl and dibenzoyl *O,O'*-diesters of apomorphine were synthesized by a procedure reported previously [4,10]. The details of the synthesis, proof of structure and purity of the compounds have been reported by Borgman *et al.* [10]. 10-OH- and 10-methoxyaporphine had been

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synthesized by Dr. Borgman's laboratory [11]; *N*-*n*-propyl-norapomorphine (10,11-hydroxylated), 10-OH and 11-OH-*N*-*n*-propylnoraporphine, 2,10,11-tri-OH-aporphine, and apocodeine (the 10-methoxy,11-OH-analogue of apomorphine) were kindly donated by Professor John Neumeyer and Dr. Felix Granchelli of Northeastern University. Labeled [6-^{3}H]-3′,5′-cyclic adenosine monosphosphate (cyclic AMP) was purchased from New England Nuclear. Bovine adrenals for preparing a binding protein used in the assay of adenylate cyclase activity were obtained from Pel-Freeze. Spectral grade ethyl acetate was purchased from Fisher Scientific. Triorthocresylphosphate (TOCP) was from Eastman; physostigmine (eserine) sulfate and *S*-adenosyl-L-methionine iodide were from CalBiochem; pyrogallol and disulfiram were from Sigma. All other chemicals were obtained from commercial sources in the highest available purity. Silica gel-on-plastic thin-layer chromatogram (t.l.c.) sheets were purchased from Eastman. Male Sprague–Dawley rats (180–220 g) and male CD-1 mice (30–45 g) were obtained from Charles River. Animals were maintained on Purina laboratory chow for rats, mice and hamsters, and water *ad lib.*

Apomorphine and its esters were dissolved for injection in a vehicle containing (by vol.) ethanol (22.4 per cent), polyethylene glycol 400 (U.S.P. grade, J. T. Baker, 52.1 per cent) and isotonic saline (25.5 per cent). The drugs were prepared at the shortest possible time before administration by first dissolving in warm ethanol, followed by addition of the other solvents; these solutions were nitrogen-gassed and kept on ice protected from light to minimize decomposition—all as described previously [4, 9]. The aporphines were injected into mice intraperitoneally (i.p.) in volumes less than 0.5 ml.

Brain apomorphine levels (using mice instead of rats in order to conserve ester) were estimated by the fluorescence method of Van Tyle and Burkman [12], except for the modification of tissue extraction methods suggested by Von Voigtlander *et al.* [13] (0.4 N perchloric acid homogenates of the tissue were extracted with 1.67 vol. ethyl acetate). Samples were read at the experimentally determined optimal excitation/emission wavelengths of 276/380 nm in an Amino–Bowman spectrofluorometer. "Blanks" and standards were prepared for each experiment in 0.4 N perchloric acid containing 0, and 1–10 μg of authentic apomorphine, respectively, and carried through the extraction and assay procedures, with and without the addition of brain tissue homogenized in the perchloric acid. As recovery of the amounts of apomorphine encountered in these experiments did not decrease appreciably when brain tissue was present (> 95 per cent recovery of authentic apomorphine), the results were not corrected for recovery of apomorphine.

Ester-hydrolyzing activity of rat tissues *in vitro* was evaluated by recovery of apomorphine fluorescence in 5.0 ml ethyl acetate (as described by Von Voigtlander *et al.* [13]) from 50- to 150-μl portions of reaction mixtures which had been diluted with 3.0 ml of 0.4 N perchloric acid after incubation of serum or 50,000 *g* supernatant fractions of homogenates of rat tissues in 2 vol. of phosphate buffer (10 mM, pH 6.6) under the conditions described in the tables and figures below. Blanks (0 μg) and recovery standards (1–10 μg) of authentic apomorphine in each experiment were prepared from reaction mixtures containing tissue extract without added ester.

Identification of apomorphine recovered from incubations with rat tissue *in vitro* or from mouse brain *in vivo* was confirmed by chromatographing ethyl acetate extracts (using the extraction methods of Erhardt *et al.* [14]) of incubation mixtures or tissue homogenates with authentic apomorphine and esters on the Silica gel t.l.c. sheets. The chromatograms were developed 9 cm in the following solvent mixtures (v/v): benzene–methanol (4:1) [14], or chloroform–methanol–ethyl acetate (92 : 5 : 3); and aporphines were visualized by the following reagents: iodine vapor, or diazotized sulfanilic acid (DSA) spray reagent [14], or by a 0.1% (w/v) solution of *N*,2,6-trichloro-*p*-benzoquinone-imine (J. T. Baker, TCBI) dissolved in chloroform–dimethylsulfoxide (DMSO) (9 : 1, v/v), using DMSO which had previously been saturated with powdered crystalline sodium bicarbonate [15].

Adenylate cyclase activity in response to dopamine or the aporphines was estimated in homogenates of striatal tissue from rat brain by the methods of Clement-Cormier *et al.* [16] and Brown *et al.* [17], with slight modification [4]. In some experiments, aliquots of reaction mixtures containing products of the hydrolysis of apomorphine esters by rat liver minces were used to evaluate the formation of an agonist of dopamine- and apomorphine-sensitive adenylate cyclase. Thus, rat liver minces (1g/4 ml of medium) were preincubated for 15 min at 37° or 4° in phosphate buffer (10 mM, pH 6.0) containing isotonic sucrose (0.32 M), apomorphine esters (0.8 mM), and pyrogallol (0.1 mM) to inhibit catechol-*O*-methyltransferase (COMT) [18]. After centrifuging (1000 *g*), aliquots (10 μl) of supernatant yielding final aporphine concentrations of 16 μM were incubated with rat striatal homogenates to assay the activity of adenylate cyclase [4, 16, 17] as already described. In some experiments the preincubation with liver mince was conducted without pyrogallol and with added methylation co-substrate, *S*-adenosylmethionine (0.1 mM).

All data are expressed as means ± S.E.M. and statistical significance of differences is evaluated by Student's *t*-test (2-tailed). Kinetic data were expressed according to Lineweaver and Burk [19] and analyzed by linear regression analysis by the method of least squares with the aid of a Hewlett–Packard programmable calculator (model 9800-10).

RESULTS

Fluorescence assays. Apomorphine produced excitatory/emission spectral maxima of 276/380 nm (Fig. 1) after calibration of the spectrofluorometer with quinine, whether apomorphine was dissolved in ethyl acetate equilibrated with either a strongly acidic or a neutral aqueous medium. These results accord well with previous studies of the fluorescence characteristics of this compound, in which values of 270/370 nm [12], or 282/379 nm [13] were reported for the same parameters. The recovery of apomorphine from liver or brain homogenates, acidified with 0.4 N perchloric acid and extracted into ethyl acetate, was vir-

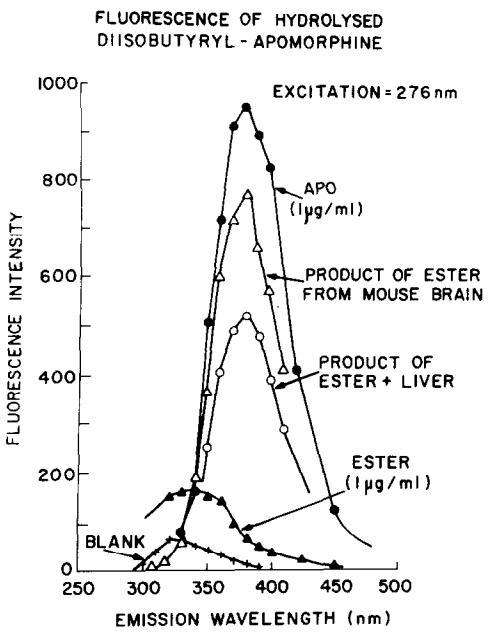

Fig. 1. Fluorescence of apomorphine and di-isobutyryl apomorphine. Fluorescence spectra were evaluated for solutions of apomorphine (APO) (●——●) or its ester (▲——▲) at the same concentration (1 μg/ml) in ethyl acetate and expressed as fluorescence units vs emission wavelength (nm), with excitation at 276 nm. In addition, the spectra are shown of an ethyl acetate extract of rat liver homogenates (○——○), acidified with 0.4 N perchloric acid after incubation with the di-isobutyryl ester (at 16 μM for 15 min at 37°), as described for Table 4; and of the product recovered, as described in Materials and Methods and for Fig. 3, from mouse brain 20 min after injection of di-isobutyrylapomorphine (120 mg/kg, i.p.) after administration of pyrogallol (250 mg/kg, i.p.) 60 and 30 min previously to prevent *O*-methylation (△——△). Blank was determined with ethyl acetate washed with perchloric acid. Data are typical values from one of three separate experiments.

tually quantitative up to 1000 ng/ml (Fig. 2), and the assay method reliably detected less than 25 ng apomorphine. Under the conditions of assay using tissue extracts, the emission peak at 380 nm with 1 μg apomorphine was consistently more than 45-times higher than the fluorescence in a reagent blank (ethyl acetate equilibrated with perchloric acid), a tissue blank (ethyl acetate extract of brain or liver homogenized in phosphate buffer with perchloric acid added) (Fig. 1), or with esters of apomorphine; the di-isobutyryl ester, which was used in most experiments, did not yield fluorescence at 380 nm appreciably above blanks, even at concentrations as high as 1000 ng/ml (see Fig. 1).

Before this assay technique was used to estimate the hydrolysis of apomorphine diesters to the free 10,11-catechol product, apomorphine, it seemed well to question whether phenolic aporphines, which would be produced by the removal of only one ester group, or the metabolism of apomorphine to apocodeine by COMT [18], have fluorescence characteristics identical to those of apomorphine. When a series of aporphines with hydroxyl or methoxy moieties in the 10 or 11 position was evaluated, none had fluorescence characteristics closely similar to those of apomorphine (Table 1). Interestingly, even slight changes in the structure of the aporphine system far from the region of a free 10,11-catechol group (*viz.* *N*-*n*-propyl-substitution to replace the *N*-methyl group, or the addition of a hydroxyl group in the 2-position) can evidently modify the fluorescence spectra (Table 1).

The assay of apomorphine by its native fluorescence was sufficiently sensitive and apparently sufficiently specific to permit the estimation of free apomorphine in tissue incubation mixtures containing apomorphine esters, or in brain after administration *in vivo* of apomorphine or its esters. For example, when the di-isobutyryl ester of apomorphine was reacted with a 50,000 *g* supernatant fraction of rat

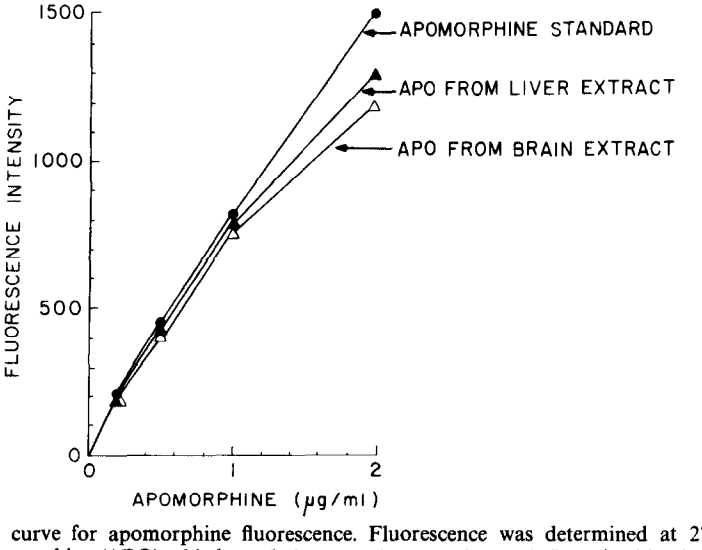

Fig. 2. Standard curve for apomorphine fluorescence. Fluorescence was determined at 276/380 nm for authentic apomorphine (APO) added to ethyl acetate (expressed as μg/ml), or for identical amounts added to homogenates of liver or brain and extracted in ethyl acetate as for Table 5. The recovery was nearly 100 per cent at least to 1 μg/ml, and the assay can detect less than 25 ng apomorphine (fluorescence of 1 μg was more than 45 times above reagent or tissue blanks). Data are means of triplicate determinations, less an appropriate blank.

Table 1. Fluorescence wavelength maxima of hydroxy or methoxy aporphines*

Compound	Excitation (nm)	Emission (nm)
Apomorphine	276	380
Apocodeine (10-methoxy,11-OH-aporphine)	318	362
2,10,11-Tri-OH-aporphine	318	369
10-OH-aporphine	320	354
10-Methoxy-aporphine	318	350
N-n-propylnorapomorphine	318	380
10-OH-*N-n*-propylnoraporphine	320	360
11-OH-*N-n*-propylnoraporphine	318	342

* All compounds were read at a concentration of 1 μg/ml in spectral grade ethyl acetate shaken with 0.4 N perchloric acid, and all produced detectable fluorescence at their optimal wavelengths, in excess of a reagent blank. When quinine was used as a standard to calibrate the peak spectral readings, its excitation and emission peaks were within ±1 per cent of published values, and so the values reported are uncorrected. The experimental variance of the reported values was not more than ±2 nm.

liver, a product was recovered by extraction into ethyl acetate after the addition of 0.4 N perchloric acid that exhibited identical fluorimetric spectral characteristics to those of authentic apomorphine (Fig. 1). This result suggested that the esters of apomorphine might be hydrolyzed in tissues to produce free apomorphine.

A fluorescent product with emission and excitation spectral characteristics identical to authentic apomorphine could be detected in brain extracts when apomorphine was given to mice 10 or 15 min previously (Fig. 3). Furthermore, the product recovered after administration of di-isobutyrylapomorphine *in vivo* had identical fluorimetric characteristics to authentic apomorphine and to the product recovered after administration of apomorphine (Fig. 1). The sensitivity of the fluorimetric tissue assay of free apomorphine was not sufficient to permit its detection in individual mouse brains after minimum behaviorally effective doses of

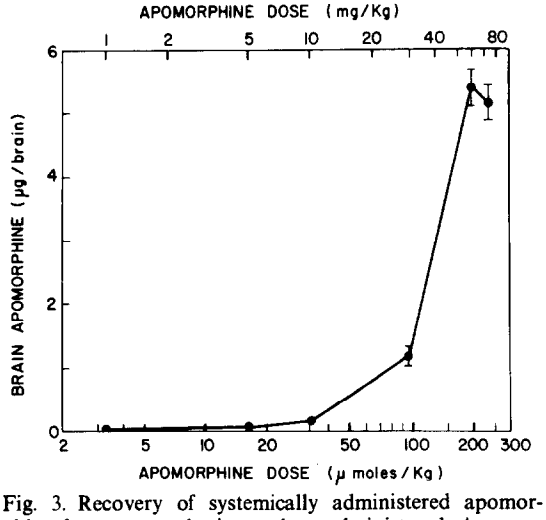

Fig. 3. Recovery of systemically administered apomorphine from mouse brain vs dose administered. Apomorphine was given i.p. in doses up to 0.25 m-mole/kg (78 mg/kg). After 15 min, brains were removed and homogenized in 0.4 N perchloric acid, extracted in ethyl acetate, and assayed fluorimetrically at 276/380 nm as described in Materials and Methods. Data are mean values ±S.E.M. for N ≥ three mice; doses of apomorphine are expressed as μmoles/kg (lower abscissa scale) and as mg/kg (upper scale).

apomorphine or its di-isobutyryl ester; nevertheless, tissue levels of apomorphine could be detected after doses (30–50 μmoles/kg) (Fig. 3) close to the estimated ED_{50} (25 μmoles/kg), rising to maximum tissue levels (Fig. 3) that corresponded to maximally effective doses (ED_{100} = 100–150 μmoles/kg) for stereotyped behavior observed in the mouse with apomorphine or the di-isobutyryl ester (R. Baldessarini, N. Kula, K. Walton and R. Borgman, *Psychopharmacology*, in press). When equimolar doses of apomorphine or its di-isobutyryl ester were given systemically to the mouse, the increase of apparent apomorphine fluorescence after administration of the ester was somewhat less in maximum amplitude than after apomorphine, although apomorphine fluorescence could be detected much longer after injection of the ester (more than 6 hr after the ester vs 60–90 min after apomorphine) (Table 2). These results are consistent with the hypothesis that the esters of apomorphine are hydrolyzed *in vivo* to provide free apomorphine, which can reach receptor sites in the brain slowly to exert prolonged neuropharmacological effects.

Independent identifications of apomorphine. Thin-layer chromatography was used to confirm the identity of the apomorphine recovered from tissues or incubations. Authentic apomorphine, when dissolved in pure ethyl acetate, migrated on Silica gel t.l.c. plates in benzene–methanol to an apparent R_f of 0.38, in close agreement with a recently reported value of 0.35 obtained with the same technique [14]; the di-isobutyryl ester ran to an R_f of 0.78 under the same conditions. When tissue extracts were used, these solvent systems produced excellent separation of free apomorphine and its esters (e.g., the R_f values for apomorphine and di-isobutyryl-apomorphine were 0.22 and 0.60 in chloroform–methanol–ethyl acetate vs 0.49 and 0.78 in benzene–methanol). Ethyl acetate extracts of mouse brain tissue after administration *in vivo* of di-isobutyrylapomorphine (100 mg/kg, i.p., 10 min previously), or of rat brain or liver homogenates incubated with this ester *in vitro* at 0.4 mM for 15 min at 37° contained a spot on Silica gel t.l.c. sheets which coincided in R_f with authentic apomorphine in both solvent systems. Moreover, the colors of the visualized spots corresponding to apomorphine and its esters were identical to those produced by the authentic compounds with all three visualizing re-

Table 2. Time course of recovery of apomorphine from mouse brain after administration of apomorphine or di-isobutyrylapomorphine*

Time (min)	Brain apomorphine (μg/brain)	
	Apomorphine	Di-isobutyryl-APO
0	0	0
10	4.34 ± 0.90	2.58 ± 0.20
20	4.70 ± 0.20	2.44 ± 0.85
60	0.37 ± 0.10	0.39 ± 0.12
90	0.04 ± 0.02	0.38 ± 0.07
240	0	0.27 ± 0.06

* Apomorphine (APO) or its ester was given at a dose of 0.25 m-mole/kg, i.p., and brains were removed, frozen on dry ice, homogenized in 0.4 N perchloric acid, extracted in ethyl acetate, and assayed for apomorphine fluorescence as described in Materials and Methods. Data are mean values of brain apomorphine ±S.E.M. (N = four to eight mice/condition).

agents used: e.g. iodine vapor gave a green reaction with apomorphine, and a "burnt orange" color with di-isobutyrylapomorphine; the DSA reagent gave gray-green and tan-gray respectively; the TCBI reagent produced blue-purple and bright green fading to blue-gray, respectively, with these two aporphines.

To provide further evidence for the presence of free apomorphine after incubation of tissue with a diester, we preincubated di-isobutyrylapomorphine with rat liver minces and then, after centrifugation, incubated portions of the supernatant reaction mixture with rat striatal homogenates containing an adenylate cyclase stimulated by dopamine or apomorphine, with an inhibitor of phosphodiesterase [4, 9, 16]. In this experiment, apomorphine led to an increase in the formation of cyclic AMP when added directly to the striatal homogenate, whereas the di-isobutyryl ester did not (even when added at 100 μM, cyclase activity = 102 per cent of control), as we have reported previously [9]; apomorphine retained most of this activity after incubation with rat liver, while the ester became active in the cyclase assay only after preincubation with tissue, but no longer did so when the preincubation was conducted on ice rather than at 37° (Table 3). Furthermore, when the COMT inhibitor pyrogallol was not included in the preincubation mixture, and the methyl donor *S*-adenosylmethionine was added, the subsequent stimulation of adenylate cyclase by the product of the ester was reduced from 145 per cent (Table 3) to 125 per cent. These results are consistent with the hypothesis that the ester was hydrolyzed to the free catechol, and that this reaction was temperature dependent, and possibly enzymatic in nature.

Characterization of ester hydrolysis. In order to pursue further the possibility that enzymatic hydrolysis of the esters can occur, an ester of apomorphine was incubated with a 50,000 *g* supernatant fraction of rat liver, and apomorphine was recovered by extraction into ethyl acetate for fluorimetric assay (Table 4). Under these conditions, apomorphine was produced by a reaction that was found to be highly dependent on temperature and tissue concentration, and to be inhibited by TOCP, a compound previously reported to inhibit non-specific esterases [9, 20], with little inhibitory effect obtained with physostigmine, an inhibitor of acetylcholinesterase (Table 4), but no inhibition with disulfiram (data not shown), a compound previously reported to have some anti-carboxylesterase activity [21]. A study of the tissue distribution of the presumed esterase activity indicated that almost all of the activity in crude homogenates of liver could be accounted for in the soluble 50,000 *g* supernatant fraction. The activity was very high in rat plasma (but low in human plasma) and was found in varying levels of activity in most other rat tissues, including low activity in brain (Table 5).

The time course of hydrolysis of a series of esters of increasing molecular weight suggested that the rate of the reaction bears an inverse relation to the size of the ester substituent (Fig. 4), under conditions in which the solubility of the esters did not seem to be

Table 3. Effect of apomorphine or hydrolyzed apomorphine ester on rat striatal adenylate cyclase*

Drug	Adenylate cyclase activity		
	Added directly to cyclase	Added after preincubated with rat liver mince	
		37°	4°
Di-isobutyryl-APO, 16 μM	89.0 ± 4.4	144.8 ± 10.1†	106.4 ± 3.5
APO, 4 μM	137.0 ± 3.4†	123.7 ± 3.9†	128.0 ± 7.3†

* Data are adenylate cyclase activity as per cent of control ±S.E.M.; N = ≥ 5; 100 per cent = 34.4 pmoles cyclic AMP/min/mg of tissue. Medium recovered from incubation with rat liver mince alone had no effect (103.7 ± 1.9 per cent), nor did ester up to 100 μM (102.0 ± 7.0 per cent) (N = 6). APO = apomorphine.

† $P < 0.01$ compared to 100 per cent value.

Table 4. Characteristics of conversion of di-isobutyrylapomorphine to apomorphine by liver*

Enzyme (mg tissue/assay)	APO (nmoles produced/15 min)	% of Control
0	0	0
10	232 ± 13	27.4
20	325 ± 27	33.4
40	511 + 29	60.4
100 (Control)	846 ± 11	100
100 + 0.1 mM TOCP	383 ± 8	45.3†
100 + 0.1 mM physostigmine	644 ± 11	76.1
100 at 4°	31 ± 1	3.6†

* *O,O'*-di-isobutyrylapomorphine (0.4 mM) was incubated with 1.0 ml of a 50,000 *g* rat liver supernatant diluted to produce the stated concentrations of tissue per assay, for 15 min at 37° in final volume at 2.5 ml; 50-μl aliquots were acidified with 3.0 ml of 0.4 N perchloric acid, and apomorphine (APO) was extracted into ethyl acetate and assayed fluorimetrically, all as described in Materials and Methods; data are means ± S.E.M. (N = 3). TOCP is triorthocresylphosphate.

† $P < 0.01$, compared with control.

a factor. When the reaction was conducted with varying concentrations of several substrate esters of increasing size, the reaction appeared to follow saturable Michaelis–Menten kinetics, consistent with the proposed enzymatic nature of the process. The apparent K_m values for several esters in at least three separate experiments clustered in the range of 0.4 to 0.8 mM, with no consistent relationship to the size of the esters, while the apparent maximum initial velocity (V_{max}) bore an inverse relationship to the size of the esters (Table 6).

DISCUSSION

In our previous studies, we have found that diesters of apomorphine exert behavioral effects identical to those of apomorphine but prolonged in proportion to the size of the ester substituent [4, 9, 10]. These behavioral effects are believed to be mediated at least in part by agonism of dopamine receptors in the CNS by apomorphine [9, 22]. Evidence for this view is that a dopamine-sensitive adenylate cyclase in rat striatal tissue is stimulated by apomorphine, but not its esters [4, 9] (see Table 3); this reaction has been proposed to reflect the stimulation of central dopamine receptors [16, 23]. The lack of effect of apomorphine diesters on the apomorphine-sensitive brain cyclase strongly suggests that they must first be hydrolyzed to the free catechol. This view accords well with the growing evidence that free catechol groups are required for aporphine compounds to exert effects *in vivo* believed to be indicative of stimulation of central dopamine receptors [see review in Ref. 9]. Thus, it is likely that the esters of apomorphine serve as "latent" drugs or prodrugs [24] of the presumed active metabolite, apomorphine.

Table 5. Conversion of di-isobutyrylapomorphine to apomorphine*

Tissue	APO (nmoles/hr)
Plasma (rat)	3666 ± 110
Gut	2160 ± 104
Heart	1772
Spleen	1620
Kidney	1517 ± 57
Liver	1022 ± 56
Muscle	468 ± 18
Brain	349 ± 19
Plasma (human)	302 ± 29

* Di-isobutyrylapomorphine (0.4 mM) was incubated with 1.0 ml of a 50,000 *g* tissue supernatant equivalent to 100 mg tissue (or with 0.2 ml plasma), in a final volume of 5 ml (1 ml for plasma) for 15 min at 37°; apomorphine (APO) was recovered and assayed as described for Table 4; data are means ± S.E.M. (N = 3) or mean values from one experiment as nmoles APO produced per mg wet wt of tissue or per ml of plasma, per hr.

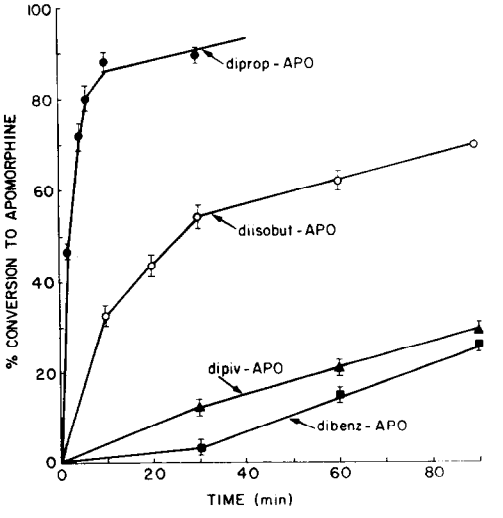

Fig. 4. Time course of hydrolysis of apomorphine esters. The following esters of apomorphine (0.4 mM) were incubated with rat liver supernatant at 37° as described for Table 4: dipropionyl (diprop-APO, ●——●), di-isobutyryl (di-isobut-APO, ○——○), dipivaloyl (dipiv-APO, ▲——▲), and dibenzoyl (dibenz-APO, ■——■); the increasing molecular weights of the free bases are: 379, 407, 435, and 375 respectively. Data represent time course as mean (± S.E.M.) per cent conversion of esters to free apomorphine as estimated after acidification with 0.4 N perchloric acid and extraction into ethyl acetate by measurement of fluorescence at 276/380 nm, as described in Materials and Methods (N = six assays).

Table 6. Maximum velocity of hydrolysis of apomorphine esters by rat liver extract *in vitro**

Ester	Mol. wt	V_{max} (nmoles APO/min)
Dipropionyl-APO	379	1088.0 ± 106.6
Di-isobutyryl-APO	407	56.0 ± 1.2
Dipivaloyl-APO	435	36.0 ± 0.6

* The esters (as HCl salts, with mol. wt given for the free base) were incubated at concentrations from 0.075 to 1.20 mM with rat liver extracts for 15 min for the larger esters and 2 min for dipropionyl-APO at 37°; reaction mixtures were then assayed fluorimetrically for apomorphine (APO) as described for Table 4. The apparent V_{max} was computed by linear regression analysis of the Y-intercept of the plot of $(\text{velocity})^{-1}$ vs $[\text{ester}]^{-1}$. Data are mean values of apparent V_{max}/0.1 g of tissue ± S.E.M. for at least three experiments. In all experiments, the data followed saturable (linear) Michaelis–Menten kinetics closely ($r > 0.98$). The apparent K_m values were all in the range of 0.4 to 0.8 mM.

Further evidence that is consistent with this proposed interpretation of the effects of apomorphine esters is that their systemic administration results in a much more prolonged appearance in the mouse brain of what seems to be free apomorphine than occurs after injection of apomorphine itself (Table 2). In addition, we have extended our behavioral findings with the rat [4, 9, 10] to the mouse, in which stereotyped cage-climbing behavior induced by apomorphine and antagonized by neuroleptic dopamine antagonists [13] was produced by di-isobutyrylapomorphine, but with a striking prolongation of action that corresponded closely with brain levels of free apomorphine (R. Baldessarini, N. Kula, K. Walton and R. Borgman, *Psychopharmacology*, in press). We have also noted that increased brain levels of apomorphine in the mouse was associated with increased levels of cyclic AMP in the striatum (a tissue found to contain dopamine- and apomorphine-sensitive adenylate cyclase in the mouse) after rapid tissue fixation [25] with focused microwave irradiation (K. Walton, N. Kula and R. Baldessarini, unpublished observations).

The present findings are consistent with the view that decreased rates of hydrolysis contribute to the increased behavioral actions of apomorphine esters in the rat or mouse, and suggest that metabolic hydrolysis of the esters is similar in these two species. Thus, evidence was presented that the esters can be hydrolyzed to apomorphine by rat or human plasma or soluble supernatant fractions of many rat tissues (see Table 5). This process was dependent on temperature and tissue concentration, and was inhibited by TOCP, an agent previously reported [20] to inhibit esterase activity (see Table 4). This time course of hydrolysis (see Fig. 4), and the apparent maximum initial velocity (see Table 6) of hydrolysis of a series of apomorphine diesters were found to be inversely related to the size of the ester substituent. Very similar findings have been reported regarding the rate of hydrolysis of a series of esters of the diphenolic branchodilator amine, terbutaline, with which the rate of hydrolysis (and V_{max} of the reactions) decreased with increasing size of the ester substituents [26].

In addition to the correlation of the rate of hydrolysis of the esters *in vitro*, with the duration of availability of apomorphine to the brain *in vivo*, and with the duration of behavioral activity of the apomorphine esters, an additional factor contributing to this extended tissue half-life and duration of activity are the possible changes in distribution, availability, and metabolism ("depot" behavior) of the esters. Thus, since the partitioning of the esters of apomorphine between organic solvents and water in the lipophilic direction appeared to increase with the bulk of the ester substituents [9], it is possible that the increased lipophilic character of the larger esters contributes to their prolonged activity. Moreover, the presence of ester groups should decrease peripheral metabolism, and presumably inactivation, of apomorphine by previously proposed mechanisms that include conjugation or methylation of the hydroxyl groups [18], thus enhancing the availability of apomorphine to the brain. The available information does not permit a clear choice between the two alternative hypotheses, nor does it exclude the possibility that both the depot characteristics and the decreased rates of hydrolysis to the active metabolite may contribute to the time course of action of the esters *in vivo*.

The availability of a mechanism by which to increase the duration of action of apomorphine has considerable potential clinical importance. The clinical use of apomorphine [6] and *N-n*-propylnorapomorphine (NPA) [27] in patients with Parkinson's disease has been severely limited by the short duration of action of these catechol-aporphines. Furthermore, diurnal or hourly fluctuations in the clinical responses to L-dopa in such patients ("on–off" phenomena) might be minimized by the availability of longer acting dopamine agonists [28]. Additional clinically desirable characteristics of the centrally active aporphines which act as dopamine agonists include increased oral activity [9] (increased gastrointestinal absorbtion and decreased peripheral metabolism), which might also be possible to achieve by the use of esters (to block metabolism) with an improved balance of lipophilic/hydrophilic properties. An ideal dopamine agonist would also avoid the nausea and vomiting that is often encountered early in the clinical use of L-dopa or apomorphine in high doses; conceivably, the lower average brain levels of apomorphine after a dose of its esters might diminish this problem. Long-acting, orally effective central dopamine agonists might be useful for conditions other than Parkinson's disease, including the management of carcinoma of the breast and certain endocrine dysfunctions by the suppression of prolactin output, which can be effected by apomorphine [29], as well as by

esters of apomorphine [8] (A. Boyd, R. Baldessarini, S. Reichlin and R. Borgman, unpublished observations in the rat). In addition, the reported paradoxical anti-dyskinetic and sedative effects of apomorphine in man (see review in Ref. 9) might also be exploited to clinical advantage with the development of labile prodrugs with prolonged activity.

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