Br. J. Pharmac. (1983), **78**, 395–404

Interactions of dopamine and the release of [^{3}H]-taurine and [^{3}H]-glycine from the isolated retina of the rat

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1 The dose-related, calcium-dependent, potassium-stimulated release of preloaded [^{3}H]-dopamine from the superfused rat retina has been demonstrated.

2 A high-affinity uptake system for dopamine exists in rat retina *in vitro*; K_m value was calculated as 1.89 μM, V_{max} value as 1.4 nmol g^{-1} tissue h.$^{-1}$

3 Dopamine (0.8 and 4 mM) inhibited the spontaneous release of [^{3}H]-glycine from retina, and in the case of 0.8 mM dopamine this inhibitory effect was antagonized by 10 μM (+)-butaclamol but not by 10 μM (−)-butaclamol.

4 The potassium-evoked (25 mM) release of [^{3}H]-glycine from rat retina was similarly inhibited by dopamine (0.4–4 mM) in a dose-related manner when added to the superfusate with the potassium. The effect of 0.8 mM dopamine was antagonized by 10 μM (+)-butaclamol but not by 10 μM (−)-butaclamol.

5 Dopamine (4 mM) significantly reduced the spontaneous release of [^{3}H]-taurine from rat retina.

6 The potassium-stimulated (25 mM) release of [^{3}H]-taurine occurred after the cessation of the depolarizing stimulus. This delayed release of [^{3}H]-taurine was unaffected if dopamine was applied to the superfusate at the same time as the potassium, but it was significantly reduced if dopamine (0.8 and 4 mM) was applied after the depolarizing stimulus had been removed and during the actual amino acid release phase.

7 The inhibition of K^+-stimulated (25 mM) delayed release of [^{3}H]-taurine by applying dopamine (0.8 mM) after the depolarizing stimulus was blocked by 10 μM (+)-butaclamol but not by 10 μM (−)-butaclamol.

8 The results are discussed with respect to the possible neurotransmitter role for dopamine within the rat retina, and its possible interaction with glycine and taurine.

Introduction

There is evidence to suggest that dopamine may function as neurotransmitter in the mammalian retina (see Starr, 1977; Wassenaar, 1979). Dopamine is the predominant catecholamine in the rat retina, being located in the inner plexiform and inner nuclear layers. In several species about 10% of amacrine cells contain dopamine (Ehinger, 1966; Laties & Jacobowitz, 1966; Sano, Yoshikawa & Konishi, 1968). In addition the enzymes necessary for dopamine synthesis and degradation are also present in this tissue (Mustakallio, 1967; Iuvone, Galli, Garrison-Gund & Neff, 1978). Exogenously applied dopamine is taken up into amacrine cells and can be released by a light stimulus in the cat retina (Kramer, 1971; 1976) and in the rabbit retina (Bauer, Ehinger & Aberg, 1980). Furthermore, high-affinity dopamine receptor binding sites (Schaeffer, 1980) and dopamine-stimulated adenylate cyclase activity have been demonstrated in mammalian retina (Spano, Govani, Hofmann, Kumakura & Trabucchi, 1977). From electrophysiological work, it has been shown that iontophoretically-applied dopamine inhibits the firing rate of ganglion cells (Ames & Pollen, 1969; Straschill & Perwein, 1969).

There is also substantial evidence to suggest that the amino acids, glycine and taurine, may also function as neurotransmitters in the retina (see Starr, 1977): taurine is the most abundant amino acid in this tissue (Pasantes-Morales, Klethi, Ledig & Mandel, 1972). High-affinity uptake systems have been

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demonstrated for both compounds (Neal, Peacock & White, 1973; Starr, 1977; Smith & Pycock, 1982) and both glycine and taurine may be released by appropriate stimulation (e.g. light or potassium-induced) from chick (López-Colomé, Salceda & Pasantes-Morales, 1978) and rat retina (Coull & Cutler, 1978; Kennedy & Neal, 1978; Smith & Pycock, 1982).

The aim of this study was to investigate the effects of dopamine on the release of preloaded radiolabelled glycine and taurine from the rat retina. Like dopamine, both amino acids are located in the inner (plexiform and nuclear) layers (Cohen, McDaniel & Orr, 1973; Yates & Keen, 1976; Lake, Marshall & Voaden, 1978; Pourcho, 1980; 1981) and thus provide the grounds for a study of catecholamine/amino acid interaction. Initially, however, preliminary studies were undertaken to see if dopamine itself would satisfy certain criteria denoting a neurotransmitter substance. Thus the dose-related release of preloaded [^{3}H]-dopamine by a depolarizing stimulus (KCl) and its calcium-dependency was investigated in an experimental system known to release [^{3}H]-glycine and [^{3}H]-taurine from the isolated retina of the rat (Smith & Pycock, 1982). Additional experiments demonstrated that dopamine inhibits the release of both radiolabelled glycine and taurine, and that this action is blocked by the specific dopamine receptor antagonist (+)-butaclamol.

Methods

Preparation of retinae

Male or female light-adapted Porton rats (200–300 g) were used in this study. Animals were killed by cervical dislocation, the eyes removed and the retinae dissected out over ice. The cornea was cut and the lens and vitreous humour removed allowing the retina to be teased away from the sclera. Each retina was removed, trimmed and cut in half, allowing the four half retinae from one animal to be used in each superfusion experiment.

Superfusion release studies

The method used to study the release of radiolabelled neurotransmitter was based on that of Srinivasan, Neal & Mitchell (1969). The four half retinae were placed in 1 ml oxygenated (95% O_2: 5% CO_2) Krebs Ringer bicarbonate buffer, pH 7.4, of the following composition (mM): NaCl 120, KCl 5, KH_2PO_4 1.5, $CaCl_2$ 5, $MgSO_4$ 1.5, $NaHCO_3$ 25 and glucose 10, and incubated for 20 min at 37°C with tritium-labelled dopamine, glycine or taurine (sp.act. 18, 21 and 10.5 Ci/mmol respectively, Amersham International) to give a final concentration of 10^{-8} M. Additionally the Krebs buffer also contained pargyline (50 μM, Sigma) and ascorbic acid (0.01%). Following incubation the tissue was transferred to a 4-chambered perspex perfusion block, and superfused with Krebs Ringer buffer at 37°C for 30 min at a rate of 0.5–0.7 ml per min. After this initial washout period, 12 or 16 consecutive 2 min perfusate samples were collected. In all experiments the first 4 fractions were used as pre-stimulation controls. During the next 4 fractions potassium chloride and/or dopamine hydrochloride (Sigma) was added to the superfusing Krebs solution.

In the experiments studying the release of [^{3}H]-dopamine, K^+ was added to the superfusate in concentrations of 12.5, 25 and 50 mM (final concentrations of K^+ 18.5, 31 and 56mM) for 8 min. In the case of the 50 mM KCl stimulus, the experiment was also conducted in which calcium was omitted from the Krebs Ringer buffer, and half the chambers were superfused with Krebs buffer containing 1mM ethylene diamine-tetra-acetic acid (EDTA) instead of Ca^{2+}.

To study the effect of dopamine on the release of ^{3}H-amino acids, a stimulating concentration of 25 mM KCl was used, a dose previously known to evoke submaximal transmitter release in this preparation (Smith & Pycock, 1982). In the case of [^{3}H]-glycine, dopamine (400 μM, 800 μM and 4 mM) and/or 25 mM KCl was added to the superfusate for 8 min during collection periods 5-8 inclusive. However, for [^{3}H]-taurine release, the dopamine was applied either together with the 25 mM KCl for 8 min (collection periods 5–8 inc.) or for 8 min after the potassium stimulus (collections periods 9–12 inc.). In all experiments where the effect of the (+) and (−)-isomers of butaclamol (10 μM, Ayerst Laboratories) was studied, the antagonist was included in the perfusion fluid throughout the entire experiment.

At the end of each experiment, 200 μl aliquots of the collected superfusate was taken for measurement of radioactivity by liquid scintillation counting. In addition, total tissue radioactivity was determined in each half retina by lysing the tissue in 200 μl distilled water for 15 min and adding 2 ml Unisolve E liquid scintillator (Koch-Light Lab. Ltd.).

High-affinity retinal uptake of [^{3}H]-dopamine

Retinae were dissected out, trimmed, dried, cut in half and weighed (4–6 mg) and incubated in 1 ml Krebs buffer at 37°C for 10 min. [^{3}H]-dopamine (0.2–2 μM) was added to each tube and incubation continued for a further 5 min after which uptake was stopped by rapid filtration on to Whatman GF/B filters. After washing with cold buffer, tissue radioac-

tivity was determined by liquid scintillation counting. Each concentration was run in triplicate, and tissue blanks were obtained by measuring [^{3}H]-dopamine uptake at 0°C for each concentration used.

Experimental design of release studies and presentation of data

The perfusion block contained 4 separate chambers and in each experiment a different treatment was applied to each chamber on a Latin square design, so that each experiment was repeated 4 times to obtain replication. For each experiment a control washout curve was run. In these experiments a fractional release figure (FRF) is obtained for each collection period, a FRF being the amount of radioactivity released during that collection period divided by the total radioactivity remaining in the retina at the start of that period. For each chamber the mean of the initial 4 FRFs is calculated to obtain a prestimulation mean baseline release. Each of the subsequent calculated FRFs is divided by this prestimulation mean to give the fractional release ratio (FRR). For a particular collection period (CP) the 4 replications of a particular treatment (as FRRs) were average and presented on the graphs on a logarithmic scale, as a percentage of the prestimulation release (Smith & Pycock, 1982). The calculated FRRs were used for analysis of variance after logarithmic transformation. The pooled error term was used for paired *t* tests of the treatment means where appropriate.

In the uptake studies, the means of triplicate determinations were fitted to double reciprocal Lineweaver-Burk plots by the method of least squares. The velocity of dopamine uptake is expressed as nmol g^{-1} wet weight tissue. h^{-1}.

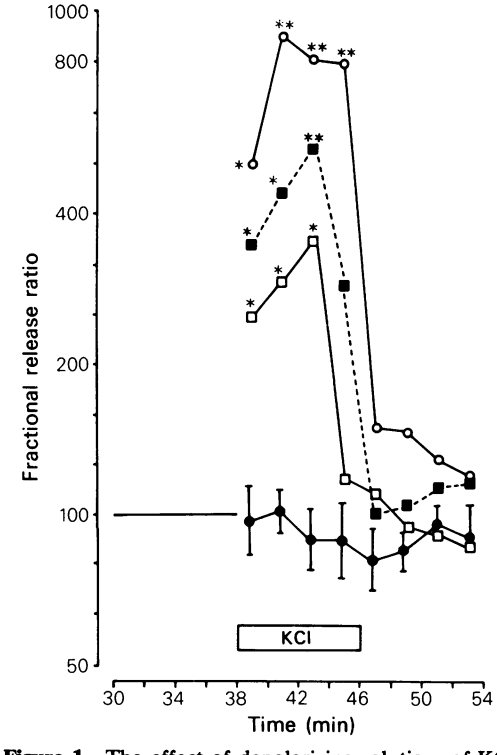

Figure 1 The effect of depolarizing solutions of KCl (applied for 8 min, as indicated by the bar) on the spontaneous efflux of [^{3}H]-dopamine from the superfused rat retina (● washout; □ 12.5 mM; ■ 25 mM; ○ 50 mM). The release is represented as a fractional release ratio, expressed as a %, on a logarithmic scale. Error bars (s.e.mean, $n = 4$) are shown on the washout curve only. All concentrations of K^+ significantly increased the spontaneous efflux of [^{3}H]-dopamine compared to washout (* $P < 0.01$; ** $P < 0.001$; Student's paired *t* test, $n = 4$).

Results

Potassium-stimulated release of [^{3}H]-dopamine

The effect of varying K^+ concentration (12.5, 25 and 50 mM) on the efflux of [^{3}H]-dopamine from the perfused rat retina is shown in Figure 1. All concentrations of potassium significantly increased the release of label (CP 6 and 7, $P < 0.01$, all 3 concentrations of K^+). The efflux associated with 50 mM K^+ was greater than that due to 12.5 mM K^+ (CP 6–8, $P < 0.05$) and to 25 mM K^+ (CP 6, $P < 0.05$).

Omission of calcium from the superfusing medium abolished the stimulatory effect of 50 mM KCl on [^{3}H]-dopamine release (CP 5–8 inc., $P < 0.01$) (Figure 2). Freeze-dried samples of superfusate co-chromatographed with authentic [^{3}H]-dopamine, indicating that 80–85% of recovered radioactivity was in the form of dopamine.

High-affinity uptake of [^{3}H]-dopamine

Radiolabelled dopamine was accumulated by rat retinae *in vitro* by a high-affinity uptake mechanism. The affinity constant was calculated as 1.89 μM and the maximum uptake velocity as 1.14 nmol g^{-1} tissue h^{-1} (from Lineweaver Burk plot, details not shown).

Effect of dopamine on the spontaneous and K^+-stimulated release of [^{3}H]-glycine

Dopamine (0.8 and 4 mM) depressed the spontaneous efflux of [^{3}H]-glycine from the superfused rat retina (CP 6–8, $P < 0.01$) (Figure 3). A lower concentration of dopamine (0.4 mM) had no significant effect on spontaneous [^{3}H]-glycine efflux

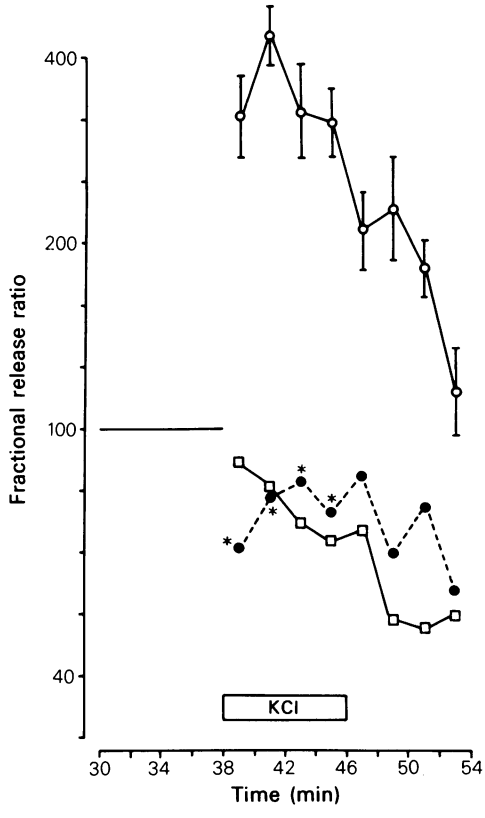

Figure 2 The effect of a depolarizing stimulus (50 mM KCl, as indicated by the bar) on the spontaneous efflux of [^{3}H]-dopamine from the superfused rat retina, in the presence (○) or absence (●) (+1 mM EDTA) of calcium. For details of representation, see the legend to Figure 1. (□) denotes spontaneous washout curve. Removal of calcium abolished K^+-stimulated release of [^{3}H]-dopamine (*$P<0.01$, comparing effect of 50 mM KCl in presence and absence of Ca^{2+}, Student's paired t test, $n=4$).

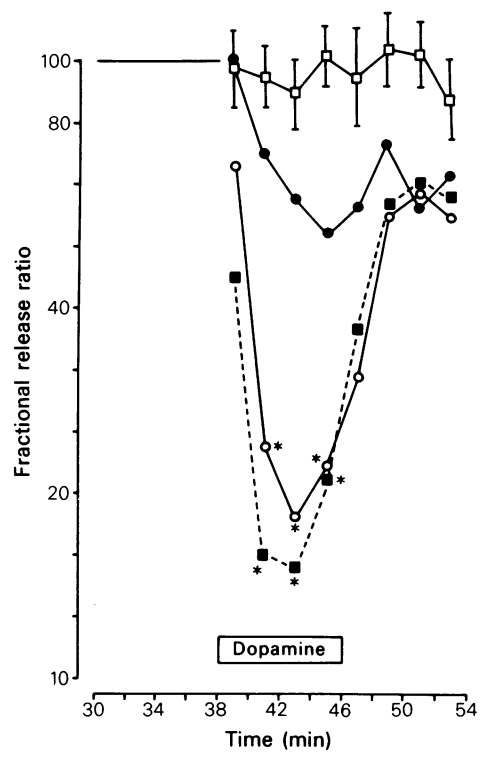

Figure 3 The effect of dopamine (● 0.4 mM; ○ 0.8 mM; ■ 4 mM) on the spontaneous efflux of [^{3}H]-glycine from the superfused rat retina. Dopamine was applied for 8 min as indicated by the bar; for details of representation, see the legend to Figure 1. (□) denotes spontaneous washout curve, and decrease in spontaneous efflux due to dopamine was compared to washout (*$P<0.01$, Student's paired t test, $n=4$).

($P>0.05$). In the presence of 10 μM (−)-butaclomol, 800 μM dopamine inhibited the spontaneous release of [^{3}H]-glycine compared to the washout curve (CP 5, 6 and 8, $P<0.05$; CP7, $P<0.01$), but in the presence of 10 μM (+)-butaclamol dopamine no longer inhibited the spontaneous efflux of radiolabelled amino acid (CP 5–12, $P>0.05$) (Figure 4).

All concentrations of dopamine (400 μM, 800 μM and 4 mM) inhibited the potassium-stimulated (25 mM) release of [^{3}H]-glycine when added to the superfusate together with the KCl (CP5, $P<0.01$; CP 6–8, $P<0.001$) (Figure 5). In fact 4 mM dopamine inhibited the spontaneous release of amino acid even in the presence of K^+. The dopamine-induced inhibition of K^+-stimulated [^{3}H]-glycine release appeared dose-related, i.e. the highest dose of dopamine producing the greatest degree of inhibition. The curves all varied significantly from each other (CP 6, and 8, $P<0.05$; CP 7, $P<0.01$).

Addition of (+)-butaclamol (10 μM) antagonized the dopamine-induced (800 μM) inhibition of K^+-stimulated [^{3}H]-glycine release (CP 5–8, $P>0.05$). In contrast (−)-butaclamol (10 μM) was without effect, and 800 μM dopamine significantly inhibited the K^+-stimulated [^{3}H]-glycine release (CP 5–8, $P<0.01$) (Figure 6).

Effect of dopamine on the spontaneous and K^+-stimulated release of [^{3}H]-taurine

The effect of dopamine (0.4–4 mM) was investigated on the spontaneous release of [^{3}H]-taurine from the perfused retina of the rat. Only 4 mM dopamine was found to decrease spontaneous [^{3}H]-taurine release

($P < 0.05$): 400 and 800 μM dopamine were without effect (Figure 7).

In order to study the effect of dopamine on the delayed K^+-stimulated release of [^{3}H]-taurine, dopamine (4 mM) was added to the superfusate either with the potassium (25 mM KCl, 8 min) or in the period immediately following cessation of the depolarizing stimulus, i.e. during the actual release phase. The results show that when dopamine was applied together with KCl the late release of [^{3}H]-taurine was unaffected (CP 9–12, $P > 0.05$; Figure 8), although during the period of application of the 4 mM dopamine the spontaneous release of radiolabelled amino acid was significantly reduced (CP 5–8, $P < 0.01$; Figure 8). However, if this concentration of dopamine were applied after the potassium stimulus, the late release of [^{3}H]-taurine was blocked (CP 9–12, $P < 0.01$, $P < 0.001$; Figure 8).

As 4 mM dopamine was considered to be rather a high concentration, this latter experiment was repeated using 400 and 800 μM dopamine applied after removal of the 25 mM KCl depolarizing stimulus. The results show that both 800 μM and 4 mM dopamine will significantly reduce K^+-stimulated release of [^{3}H]-taurine from superfused rat retina if applied after the cessation of the depolarizing stimulus (800 μM, CP 10 and 11, $P < 0.05$; Figure 9).

The inhibition of the K^+-stimulated (25 mM KCl) late release of [^{3}H]-taurine by 800 μM dopamine was antagonized by 10 μM (+)-butaclamol (CP9, 10 and 11, $P < 0.05$), but not by 10 μM (−)-butaclamol. (CP 5–16, $P > 0.05$) (Figure 10).

Discussion

The results of this study demonstrate a high-affinity uptake system for, and a dose-related potassium-stimulated release of, dopamine in the rat retina. The release of dopamine in response to 50 mM KCl appeared calcium-dependent. This, together with other experimental data (Wassenaar, 1979), provides strong evidence that dopamine should be considered for a neurotransmitter role in this tissue. The catecholamine is located mainly in the inner nuclear and inner plexiform layers (Malmfors, 1963) and in many species it is the amacrine cells which contain dopamine (see Introduction).

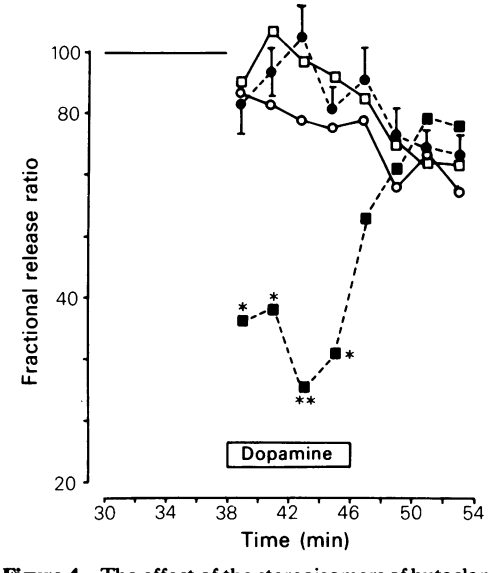

Figure 4 The effect of the stereoisomers of butaclamol on the depression of spontaneous efflux of [^{3}H]-glycine induced by 800 μM dopamine in the superfused rat retina. Butaclamol was present in the perfusing medium all the time as either (+)-butaclamol (open symbols) or (−)-butaclamol (closed symbols) in a concentration of 10 μM, and was combined in the presence (□, ■) or absence (○, ●) of dopamine applied for 8 min as indicated by the bar. For details of representation, see legend to Figure 1 (*$P < 0.05$, **$P < 0.01$, Student's paired t test, $n = 4$).

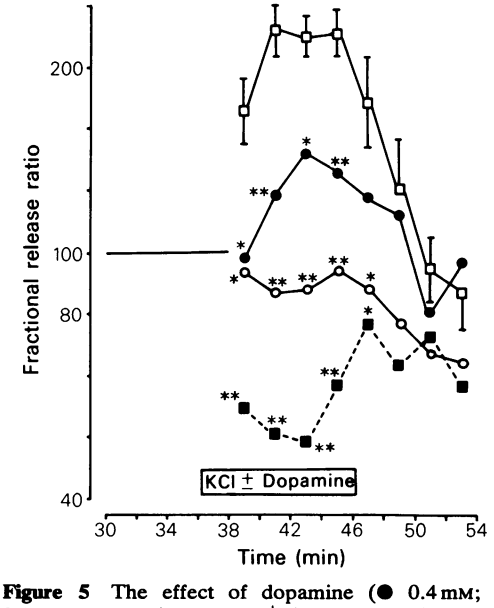

Figure 5 The effect of dopamine (● 0.4 mM; ○ 0.8 mM; ■ 4 mM) on the K^+ (25 mM, 8 min)-induced efflux of [^{3}H]-glycine from the superfused rat retina. Dopamine was included during the application of potassium only; (□) denotes the effect of K^+ in the absence of dopamine. For details of representation, see legend to Figure 1. All concentrations significantly decreased the K^+-evoked efflux of [^{3}H]-glycine (*$P < 0.01$, **$P < 0.001$; Student's paired t test, $n = 4$).

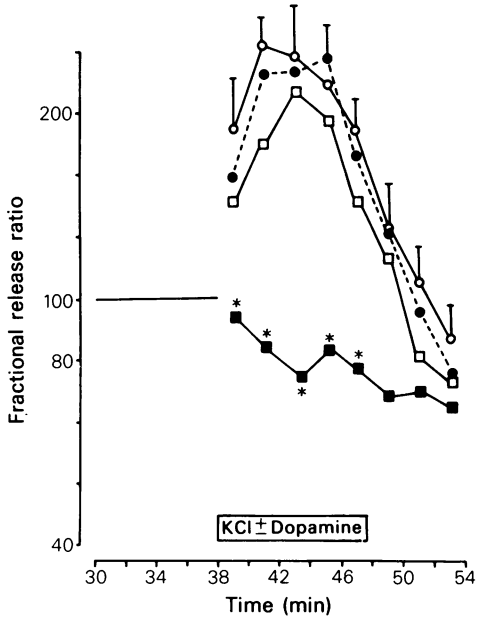

Figure 6 The effect of the stereoisomers of butaclamol on the depression of potassium-induced efflux of [^{3}H]-glycine induced by 800 μM dopamine in the superfused rat retina. Butaclamol was present in the perfusing medium all the time as either (+)-butaclamol (open symbol) or (−)-butaclamol (closed symbols) in a concentration of 10 μM, and was combined with 25 mM KCl applied for 8 min in the presence (□, ■) or absence (○, ●) of dopamine. For details of representation, see legend to Figure 1. (*$P<0.01$, Student's paired t test, $n=4$).

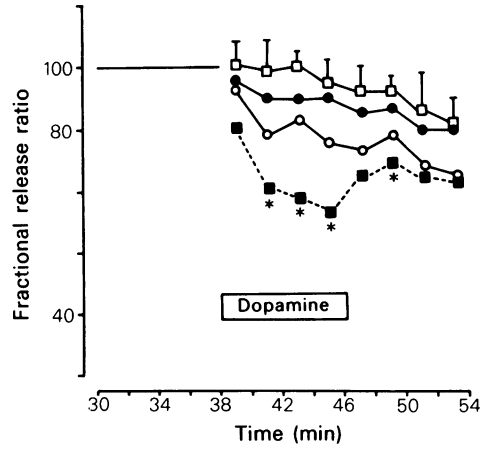

Figure 7 The effect of dopamine (● 0.4 mM; ○ 0.8 mM; ■ 4 mM) on the spontaneous efflux of [^{3}H]-taurine from the superfused rat retina. Dopamine was applied for 8 min as indicated by the bar; for details of representation, see the legend to Figure 1. (□) denotes spontaneous washout curve, and decrease in spontaneous efflux due to dopamine was compared to washout (*$P<0.05$, Student's paired t test, $n=4$).

The experimental data presented indicate that dopamine will inhibit the spontaneous efflux of [^{3}H]-glycine and the K^+-stimulated release of both [^{3}H]-glycine and [^{3}H]-taurine from the superfused rat retina. Such an action would appear to be specifically manifest through dopamine receptors, as this inhibition is blocked by the dopamine receptor antagonist, butaclamol. In particular it is the stereoactive isomer (+)-butaclamol, which selectively blocks dopamine action, and not the inactive form, (−)-butaclamol (Seeman, Chau-Wong, Tedesco & Wong, 1975). However, on a cautionary note it is worth mentioning that some of the physiological and pharmacological actions of dopamine may be mediated through α-adrenoceptors (Hurst, Marshall & Nasmyth, 1979; Baggio & Ferrari, 1981). Moreover recent experimental data have indicated that (+)-butaclamol can antagonize α_2-adrenoceptors (Spedding & Berg, 1982) and so the possibility exists that some of the effects of dopamine in the retina may be mediated, in part, by α-adrenoceptors.

There is growing evidence that both glycine and taurine may satisfy some of the criteria depicting a neurotransmitter role in the retina. Other workers have shown that glycine is specifically taken up into the inner plexiform layer in the rat retina (Marshall & Voaden, 1974) by a high-affinity uptake system (Neal *et al.*, 1973). We and others have previously demonstrated that [^{3}H]-glycine can be released from the rat retina by raising external potassium concentrations, and that this effect is calcium-dependent (Smith & Pycock, 1982). The fact that dopamine inhibits the release of radiolabelled glycine from the perfused rat retina is interesting. As both dopamine and glycine are located in subpopulations of amacrine cells, it may be predicted that dopaminergic cells may synapse on to glycine-containing amacrine cells, and thus exert some controlling influence over their function.

The role of taurine in retinal neurotransmission is less well supported. However taurine is the predominant amino acid in the rat retina (Pasantes-Morales *et al.*, 1972; Starr, 1973), and it appears to be most concentrated in the photoreceptors although significant amounts exist in all layers of the rat retina (Orr, Cohen & Lowry, 1976; Yates & Keen, 1976). A high-affinity uptake system has been demonstrated for this amino acid in this tissue (Neal *et al.*, 1973; Starr, 1978) and high external potassium concentrations have been shown to release [^{3}H]-taurine *in vitro* (Smith & Pycock, 1982). The fact that dopamine can affect the K^+-stimulated efflux of radiolabelled taurine through a presumed receptor

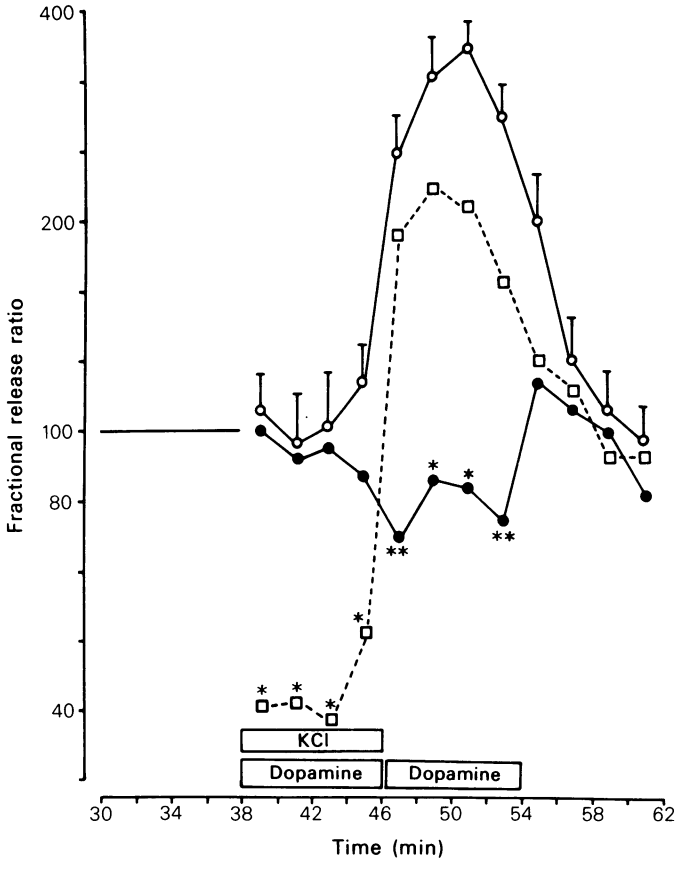

Figure 8 The effect of dopamine (4 mM) on the K^+-stimulated (25 mM, 8 min) efflux of [^{3}H]-taurine from the superfused rat retina. Dopamine was included either during the application of potassium (□, CP 5–8 inc.) or during the period immediately afterwards (●, CP 9–12 inc.); (○) denotes the effect of K^+ in the absence of dopamine. For details of representation, see legend to Figure 1. (*$P<0.01$, **$P<0.001$ compared to K^+-stimulated curve alone, Student's paired t test, $n=4$).

mechanism, and that dopamine is confined to the inner nuclear and inner plexiform layers, would suggest that taurine too is also released from these two layers of the retina. Alternatively the possibility that dopamine may affect taurine release indirectly via interneurones or bipolar cells should be considered if taurine has a role in neural mechanisms only in the photoreceptor layer of the retina. In support of the former view, it has been shown that some labelled taurine is taken up in the cat retina into a subpopulation of amacrine cells (Pourcho, 1981).

An interesting phenomenon concerning the potassium-stimulated release of [^{3}H]-taurine is that, in our system, efflux of amino acid is delayed, occurring after cessation of the applied K^+ stimulus (Smith & Pycock, 1982). In this series of experiments we have shown that dopamine only affects the increased efflux of [^{3}H]-taurine if it is applied after the K^+-stimulus, during the time when the mechanism causing the release of amino acid is active. This observation again supports the view that it is the *cessation* of the stimulus which is physiologically important for invoking the release of taurine. Such an inhibition of [^{3}H]-taurine release does not occur if the dopamine is applied *during* the period of potassium stimulation. However in contrast, no reliable effects of dopamine were observed on the very low rate of spontaneous release of radiolabelled taurine from the perfused rat retina. In this instance only the highest concentration of dopamine (4 mM) produced a significant effect, although it is worth mentioning that in keeping with the K^+-stimulated release, the action of dopamine was immediate and did not occur through a delayed mechanism. The differing effects of potassium and

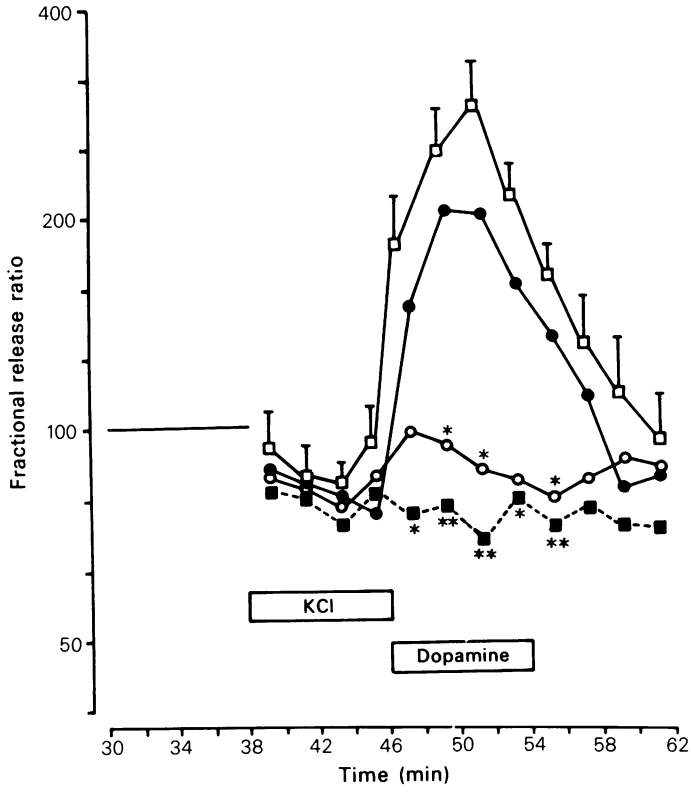

Figure 9 The effect of dopamine (● 0.4 mM; ○ 0.8 mM; □ 4 mM) on the K^+ (25 mM, 8 min)-induced efflux of [^{3}H]-taurine from the superfused rat retina. Dopamine was included in the superfusing fluid for 8 min immediately after the application of potassium; (□) denotes the effect of K^+ in the absence of dopamine. For details of representation, see legend to Figure 1. ($^*P<0.05$, $^{**}P<0.01$ compared to potassium alone curve, Student's paired t test, $n=4$).

dopamine might therefore indicate the existence of two different mechanisms capable of influencing retinal taurine release.

L.F.P.S. was supported by a vacation scholarship from the Wellcome Trust.

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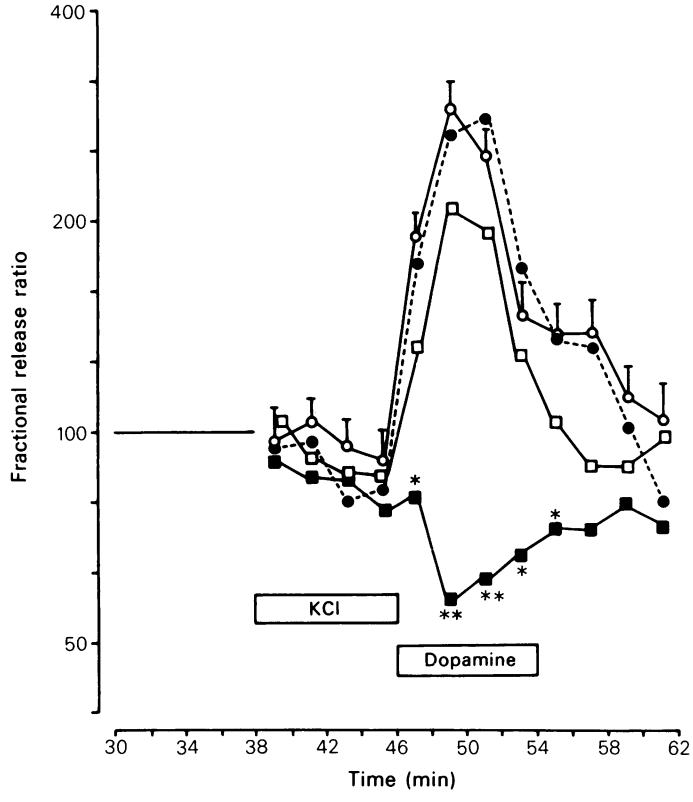

Figure 10 The effect of the stereoisomers of butaclamol on the depression of potassium-induced efflux of [^{3}H]-taurine induced by 800 μM dopamine in the superfused rat retina. Butaclamol was present in the superfusing medium all the time as either (+)-butaclamol (open symbols) or (−)-butaclamol (closed symbols) in a concentration of 10 μM, and was combined with 25 mM KCl applied for 8 min after which was applied dopamine (□, ■) or no dopamine (○, ●). For details or representation, see legend to Figure 1. (*$P<0.05$, **$P<0.01$; Student's paired t test, $n=4$).

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(*Received August 16, 1982.*)