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The Effect of Prostaglandin E_1 in Inhibiting the Pre-junctional Release of Norepinephrine in the Field-Stimulated Rabbit Iris

by

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The first experiments that seemed to indicate the presence of a substance with significant biological effects in the prostate gland of humans were made by Battez and Boulet[51] in 1913, who observed that extract of fresh human prostate tissue caused a strong depressor action on the blood pressure of dogs. In 1930, Kurzrok and Lieb[52] made the interesting observation that uteri from patients who had completed sucessful pregnancies responded with relaxation upon addition of seminal fluid, but uteri from women who had a history of complete or long standing sterility were stimulated. Shortly after this, von Euler [53] and Goldblatt [54] independently reported the smooth muscle stimulating and vaso depressor properties of human semen. Due to the occurence of this biologically active compound in extracts of the prostate and vesicular glands, it was called prostaglandin.

The observation in a variety of smooth muscle tissues of antagonistic as well as agonistic actions of prostaglandin E_1 and E_2 (PGE_1, PGE_2) on muscle contraction have stimulated more detailed investigations. Much interest has recently been focused on a role for prostaglandin in the modulation of sympathetic neuromuscular transmission.

Electrical stimulation of sympathetic nerve fibers depolarize the cell membrane, causing release of stored

norepinephrine. This norepinephrine crosses the neuromuscular junction and causes the depolarization and appropriate responses in the effector cells. It has been proposed by Hedqvist and others that endogenous prostaglandins of the E series modulate nervous transmission by a feedback mechanism, thus modifying the effector responses to nerve impulses in two ways: prostaglandin appears to inhibit the pre-junctional release of norepinephrine, and at the same time potentiate the effect of any norepinephrine present post-junctionally.

Pre-junctional Effects

Prostaglandin E_1 and prostaglandin E_2 have been shown to decrease release of norepinephrine following electrical stimulation of the splenic nerve in cats.[1,2] In a further study dealing with responses to graded doses of prostaglandin E_2 in the cat spleen, prostaglandin E_2 was found to markedly and reversibly depress the outflow of norepinephrine in response to splenic nerve stimulation.[3] (Mechanical responses of the spleen were similarly affected by low doses of prostaglandin E_2.) Furthermore, this inhibition of norepinephrine release was found to progressively increase with the concentration of prostaglandin E_2. In a spleen loaded with labeled norepinephrine, almost all of the radioactive material released by nerve stimulation consists of unchanged norepinephrine.[4] Prostaglandin E_2 caused a closely parallel reduction of the outflow of radioactively and fluorometrically determined norepinephrine, thus indicating that altered metabolic degradation of norepinephrine is of minor importance.[3]

There is further evidence that the action of prostaglandin is directly on the release of norepinephrine from the sympathetic nerve terminal. Prostaglandin E_2 does not inhibit the conduction of impulses in sympathetic nerves.[5] It is therefore unlikely that the reduced outflow of norepinephrine in the

presence of prostaglandin is due to decreased conduction of impulses. The arterio-venous difference in tritium labeled norepinephrine in the cat spleen[3] was not appreciably changed in the presence of prostaglandin E_2 in concentrations known to severely effect the outflow of norepinephrine in response to nerve stimulation. Thus it is unlikely that prostaglandin E_2 reduces the outflow of norepinephrine by facilitating re-uptake of norepinephrine released from the nerve terminals.

Prostaglandin E_1 and prostaglandin E_2 inhibit the release of norepinephrine during electrical stimulation of the guinea pig vas deferens.[6,7] The contractile response to electrical nerve stimulation is likewise inhibited in the presence of low doses of the prostaglandins of the E series.

Prostaglandin E_1 and prostaglandin E_2 progressively and reversibly inhibited the outflow of norepinephrine following sympathetic nerve stimulation in the perfused rabbit heart, as well as the inotropic and chrontropic responses. Following infusion of exogenous norepinephrine, however, the chronotropic and inotropic responses were only slightly andunpredictably effected by prostaglandin E_1 or prostaglandin E_2 within the same dose range,[8,9,10,11] thus indicating the importance of prostaglandin's pre-junctional

action. The only difference between prostaglandin E_1 and prostaglandin E_2 in the action on the rabbit heart seemed to be that prostaglandin E_1 more markedly reduced the inotropic response to nerve stimulation, whereas prostaglandin E_2 more markedly reduced the chronotropic response.

There is some evidence that prostaglandin may act pre-junctionally on cholinergically innervated tissues as well.[31] The mechanism is not yet fully understood, although it is suggested that prostaglandin acts to block the calcium influx at the axonal membrane induced by depolarization.[32, 33]

Post-junctional Effect

The prostaglandins of the E series may modulate sympathetic neurotransmission by potentiating the post-junctional effect of norepinephrine. The administration of norepinephrine directly stimulates the post-junctional effector cells. Thus, following the administration of norepinephrine, the post-junctional effect of prostaglandin can be investigated, completely excluding any pre-junctional action.

In the cat spleen, the mechanical response to nerve stimulation is modified by prostaglandin E_2 in a manner somewhat different from its effect directly on norepinephrine release.[3] Whereas the vascular pressor and capsular contractile responses were reduced in the presence of low doses of prostaglandin E_2, with

higher doses the pressor response returned to near normal, although the capsular response was further depressed. A similar pattern was seen when the nerve stimulation was replaced by administration of norepinephrine, the pressor response being moderately reduced by low doses of prostaglandin E_2 but markedly increased by high doses. This observation is in agreement with data from isolated vascular strips.[12] The results with prostaglandin E_1 are qualitatively similar.[2,3]

The contractile response to electrical stimulation in the guinea pig vas deferens[6,13] and guinea pig seminal vesicle[14] is inhibited in the presence of low doses of prostaglandin E_1 or prostaglandin E_2, but potentiated by high doses. On the other hand, contractile responses to norepinephrine are potentiated by low as well as high doses of prostaglandin E_1 or prostaglandin E_2. In addition, when electrical stimulation of the seminal vesicle was replaced by administration of norepinephrine, contractions were increased by prostaglandin E_1 or prostaglandin E_2 in doses known to reduce the response to nerve stimulation.[14] Thus the interaction of the prejunctional and post-junctional actions of prostaglandin in electrically stimulated tissue may yield biphasic results in the presence of varying prostaglandin concentrations. After

norepinephrine administration on the other hand, only the post-junctional potentiating effect of prostaglandin is present.

Site of Origin

Endogenous prostaglandin E_2 and $F_2\alpha$ are released from the dog and cat spleen during electrical stimulation of the splenic nerve or perfusion with norepinephrine or epinephrine.[15,16] It is unlikely that the source of these prostaglandins is the nerve fibers. Antagonists for alpha receptors prevent splenic contraction and also prevent release of prostaglandins in response to nerve stimulation.[15,48] In addition, the release of prostaglandins from isolated spleens which had been denervated 8 and 9 days earlier was studied.[16] These spleens contract in response to perfused epinephrine, accompanied by an output of prostaglandin. Histological examination of the stimulated tissues shows that they contain substantial nerve trunks in various stages of degeneration. Electrical stimulation of the degenerate nerve produces a slight rise in resistance to perfusion but no resultant output of prostaglandin. Increasing the perfusion pressure into the spleen, whether by infusion of vasopressin increasing flow into the spleen, or impeding

outflow from the spleen, is not accompanied by prostaglandin release. It is suggested that the prostaglandin originates in the capsular smooth muscles. Infusion of prostaglandin E_2 alone into the spleen does not produce any splenic contraction. Epinephrine similarly applied contracts the spleen and releases prostaglandin.

Further evidence of the regulatory role of endogenous prostaglandin was obtained from studies of the rabbit heart. Prostaglandin E_1 and prostaglandin E_2 are released from the rabbit heart in response to sympathetic nerve stimulation, in quantities capable of inhibiting the neuronal release of norepinephrine. The release of prostaglandin of the E series from the heart during non-stimulated conditions is much smaller, perfusate from the stimulated heart being significantly more capable of reducing heart rate and outflow of labeled norepinephrine than perfusate from the non-stimulated spontaneously beating heart. Thin layer chromatography of the perfusate confirms the presence of both prostaglandin E_1 and prostaglandin E_2.[17,18,19]

The outflow of prostaglandin E_2 in these rabbit heart preparations is not affected by the alpha blocking drug phenoxybenzamine, or the beta blocking drug propranolol, the latter in doses sufficient to completely block the mechanical

response to infusion of norepinephrine. In experiments in which the adrenergic nerves were almost completely destroyed, the outflow of prostaglandin E_2 is not significantly altered.[20] Thus, prostaglandin in the rabbit heart does not appear to be derived from the adrenergic nerves, or via receptors which are influenced by alpha or beta blocking agents. Mechanical response of the effector cell is not a prerequisite for prostaglandin release. Increased formation and release of prostaglandin E compounds in response to sympathetic nerve stimulation has also been reported in rat[21] and dog[22] adipose tissue, and in the dog kidney.[23]

Inhibitors of Prostaglandin Synthesis

Experiments using inhibitors of the synthesis of prostaglandins provide further evidence for the role of endogenous prostaglandin in modulating sympathetic nervous activity. Indomethacin, an inhibitor of prostaglandin synthesis,[24,25,26] decreases the level of prostaglandins and increases the response to epinephrine infusion in dog spleens.[25] Indomethacin is associated with increased release of labeled norepinephrine in the electrically stimulated guinea pig vas deferens[7] and increased sympathetic responses in the electrically stimulated cat spleen.[26] The chemically unrelated inhibitor of prostaglandin

synthesis, 5,8,11,14 eicosatetraynoic acid,[27,28] has been shown to increase the release of norepinephrine from stimulated guinea pig vas deferens,[29] rabbit heart,[28] and cat spleen.[30]

Experimental uptake and field stimulation-induced release of Norepinephrine

Adrenergic nerves take up and accumulate administered ^{3}H-norepinephrine *in vivo* and *in vitro*.[34,35,36] An isolated iris preparation maintained in oxygenated physiologic buffer at 37°C will retain its metabolic and electrical properties for several hours.(c.f. 37) Field stimulation of nervous tissue will cause depolarization of the nerve cell membrane and release of transmitters.[39,40,41,42,43,44,37,45,46]

Adrenergic nerves in the rat iris actively take up ^{3}H-norepinephrine and incorporate it into the amine storage granules to a very high extent.[35] Rat irides incubated in a buffer containing ^{3}H-norepinephrine release this labeled neurotransmitter from the amine storage granules when subjected to field stimulation.[37,45] More than 95% of the tritium taken up and accumulated in similarly incubated rat irides is unchanged ^{3}H-norepinephrine.[34] During field stimulation of heart and brain slices,[35] and in studies with mouse vas

deferens,[40] 70% of the tritium efflux is ^{3}H-norepinephrine. The spontaneous efflux of tritium is initially fairly rapid in these irides[37] but declines to reach a fairly stable level after 20 minutes. Under the influence of field stimulation (12 milliamps, 2 milliseconds, 10 per second) there is a marked increase in the tritium outflow. Such stimulation, continued for 60 minutes, lowers the total tritium content of the iris to 42% of that in the unstimulated control. The decrease in tritium in the stimulated iris is equal to the net stimulation induced efflux in the superfusing medium. Some areas of the adrenergic nerves when examined in the fluorescent microscope[47,37] show a moderate decrease of norepinephrine fluorescence activity with a smooth appearance of the nerve terminal and less prominent varicosities than did the unstimulated control.

When irides previously incubated with ^{3}H-norepinephrine are perfused in a buffer containing reserpine phosphate, a known inhibitor of neuronal norepinephrine uptake the results are even more pronounced.[37] There is a considerable spontaneous efflux of tritium which decreases gradually. The effect of stimulation is maintained only for the first 15 minutes and then rapidly diminishes. For the last 15 minutes of the 60 minute stimulation the norepinephrine outflow from the

unstimulated iris exceeds that of the stimulated iris. In the stimulated iris almost no fluorescent activity in the adrenergic nerve is found, in contrast to the unstimulated control iris which has weakly fluorescent varicóse nerve terminals. The tritium content of the stimulated iris decreases to 37% of the content of the unstimulated control. Thus, with the reuptake of liberated norepinephrine blocked by the reserpine, the adrenergic neurons undergo an even more pronounced depletion of norepinephrine under the influence of field stimulation. In the case of rats pretreated with reserpine and nialamide before dissection and incubation in ^{3}H-norepinephrine, there is no difference in tritium efflux or fluorescent histological appearance between the stimulated and control iris. Thus, if the capacity of the tissue to actively transport ^{3}H-norepinephrine into its adrenergic neuronal storage granules is pharmacologically blocked, field stimulation will be unable to provoke a release of the tritium labeled neurotransmitter.

In a subsequent study[42] in which a more accurate enzymatic determination of norepinephrine levels was included the above conclusions are confirmed. Field stimulation of irides from untreated rats lowers their norepinephrine content to 60% of that of unstimulated control. Fluorescent histochemical

changes in microscopic examination of the adrenergic nerves parallel the results of the enzymatic assay for norepinephrine.

It is thus apparent that field stimulation induces the release of ^{3}H-norepinephrine from the neuronal amine storage granules. This process is augmented in the presence of a drug which blocks reuptake of liberated ^{3}H-norepinephrine by the nerve terminals, and eliminated completely when the tissue is originally incubated in the ^{3}H-norepinephrine in the presence of a drug which blocks its uptake into the neuronal storage granules. The decrease of fluorescent intensity in the adrenergic nerves provides further evidence that it is neuronal norepinephrine which is released during field stimulation; in addition when no increase in tritium efflux is obtained no change in the microscopic appearance of the nerve is seen.

There thus appears to be good evidence that neuronal ^{3}H-norepinephrine is released from storage granules in the adrenergic nerves in field stimulated tissue, and that most of the stimulus induced tritium efflux in such cases represents and consists of unchanged ^{3}H-norepinephrine.

Purpose

It is becoming increasingly clear that endogenous prostaglandins play an important modulating role in sympathetic

neurotransmission in an ever widening circle of tissues and organs. Prostaglandins have been shown to inhibit pre-junctional neuronal norepinephrine release, and potentiate the effect of norepinephrine on the post-junctional effector cell.

Under the influence of electrical field stimulation, neuronal ^{3}H-norepinephrine is released from neuronal amine storage granules in previously incubated adrenergic tissues. Furthermore, most of the stimulus-induced tritium efflux in such cases represents and consists of this ^{3}H-norepinephrine.

The purpose of the present investigation was to demonstrate the pre-junctional inhibitory effect of prostaglandin E_1 on adrenergic norepinephrine release in the rabbit iris.

MATERIALS AND METHODS

General

Male albino rabbits weighing 2-3 kg were sacrificed by ether inhalation, and their eyes were rapidly enucleated. A freshly made modified Krebs-Ringer-bicarbonate buffer was available which had the following composition: 118 mM NaCl; 4.8 mM KCl; 0.33 mM $CaCl_2$; 1.2 mM KH_2PO_4; 1.2 mM $MgSO_4$;

25 mM $NaHCO_3$; 0.1 mM ascorbic acid; and 10 mM dextrose, at pH 7.4. The buffer was constantly aerated by bubbling compressed air through it. Prostaglandin E_1 (Upjohn Company, Michigan) was diluted in 10% absolute ethanol, 90% de-ionized water. It was kept frozen in 0.04 ml aliquots containing 0.002 mg PGE_1 until immediately prior to use. The prostaglandin E_1 aliquots were then diluted with warmed (37° C.) aerated buffer to form a final PGE_1 concentration of 10 ng/ml, 20 ng/ml, or 60 ng/ml in the solution perfusing the iris chamber.

Isolated Iris-Sclera Preparation

Following enucleation the eyes were placed in warmed aerated buffer solution. The posterior two-thirds of the globe was removed. The cornea was removed following trephining, by circumferential excision just on the corneal side of the limbus. The lens was gently brushed away, along with the vitreous humor, from the ciliary body, and then the remaining scleral portions were cleansed of excess uveal tissue without damage to the base of the iris. The scleral ring, with the iris attached, was mounted between two lucite o-rings by perforating and connecting needles.

Norepinephrine Uptake Phase

The iris-sclera preparation mounted in the lucite o-rings was incubated at 37° C. for 45 minutes in 5 ml of the aerated buffer solution which contained d,l-^{3}H-norepinephrine (9.4 C/mMole) (New England Nuclear Corporation, Boston, Massachusetts). The norepinephrine concentration was 8.5×10^{-8}M. In the experiments where indicated, 3×10^{-6} M cocaine sulfate was present.

Perfusion Chamber

A lucite perfusing chamber was used in the subsequent stages of the experiment. Externally its dimensions were 3.1 centimeters by 4.4 centimeters by 3.8 centimeters. The inner chamber, constructed to hold the iris-sclera o-ring sandwich was cylindrical with diameter 1.1 centimeters and height 1.3 centimeters, internal volume 1.23 cubic millimeters. Two circular platinum electrodes were present on either end of the cylinder. Aerated buffer at 37°C could be perfused through this inner chamber at a variable rate. In addition, fluid at 37°C could be perfused through the outer chamber to maintain and stabilize the temperature.

Norepinephrine Release Phase

Following the uptake phase the iris preparation was rinsed in 80 ml of warm, aerated buffer, and then transferred to the perfusing chamber. Buffer was perfused through the chamber at a rate of 2.0 ml per minute. The effluent was collected from the outflow of the chamber in one minute samples by a fraction collector. Where indicated, PGE_1 was added to the perfusing media to give a final concentration of 10 or 60 ng/ml.

0.2 ml of each collected 1 min sample was transferred to a liquid scintillation counting vial, and 10 ml of Aquasol (New England Nuclear Corporation) was added. The vials were counted for 10 min each in the Nuclear Chicago Corporation Mark II Liquid Scintillation System.

Field stimulation was accomplished with the two circular platinum electrodes inside the inner perfusion chamber on either side of the mounted iris. The stimulation was provided by a Grass SD5 stimulator at 10 pulses per second, each pulse being 18 volts and 3.2 milliseconds duration. The pulses were monophasic, and the polarity was reversed each minute. Stimulation was continued in this manner for 10 minutes.

RESULTS

When an iris, which has been previously incubated in the presence of ^{3}H-norepinephrine, is washed in the perfusion chamber with buffer, a continuously decreasing tritium efflux occurs (Figure 1). If electrical field stimulation is applied, an additional stimulus-induced tritium efflux occurs (Figure 2), creating an upward sloping portion of the curve representing a quantitative increase in the tritium efflux.

In other experiments, the iris was incubated in the ^{3}H-norepinephrine in the presence of cocaine. Active uptake of the labeled norepinephrine into the neuronal amine storage granules is presumably blocked pharmacologically. These irides were subsequently placed in the perfusion chamber and electrically field stimulated. No stimulus-induced tritium efflux occurred; the curve was continuously decreasing (Figure 3).

When prostaglandin E_1 was present in the perfusing medium at a final concentration of 10 ng/ml, no stimulus-induced tritium efflux occurred; the efflux continuously decreased (Figure 4). Thus, stimulus-induced release of norepinephrine from the neuronal amine storage granules does not occur in similar quantities in the presence of this concentration of prostaglandin E_1.

Effects of prostaglandin E_1 in concentrations of 20 ng/ml were equivocal. With prostaglandin E_1 in the perfusing medium at a final concentration 60 ng/ml, a stimulus-induced tritium efflux occured (Figure 5). Thus the inhibitory effect of prostaglandin E_1 on the neuronal release of labeled norepinephrine appears to be inversely related to its concentration, within the range studied.

DISCUSSION

We have developed a method for effectively electrically field-stimulating an isolated rabbit iris. This isolated iris, incubated in 5 ml of the 8.5×10^{-8}M ^{3}H-norepinephrine solution, is able to incorporate some of this labeled norepinephrine into its neuronal amine storage granules. With the onset of field stimulation, an increase in tritium efflux from the perfused iris occurs, representing release of ^{3}H-norepinephrine from the neuronal amine storage granules. Cocaine present in the incubating solution (3×10^{-6}M.) blocks only the active uptake of ^{3}H-norepinephrine into these granules[50], and electrical field-stimulation of irides so incubated does not result in an increase in tritium efflux. Thus, the field-stimulation-induced peak of tritium efflux represents release of ^{3}H-norepinephrine from the amine storage granules on the nerve terminals.

Hedqvist and others have proposed that prostaglandins modify sympathetic neurotransmission by a pre-junctional inhibition of norepinephrine release, and by a post-junctional alteration of the effector response to norepinephrine released. In the presence of 10 ng/ml prostaglandin E_1 in the solution perfusing the iris, no field-stimulation

induced peak of tritium efflux occured. Thus, we have shown that prostaglandin E_1 at this concentration inhibits the pre-junctional release of norepinephrine in the rabbit iris. This represents a confirmation of the pre-junctional part of Hedqvist's hypothesis for the rabbit iris. This is the first demonstration of the validity of the first part of Hedqvist's hypothesis for any ocular tissue in any species, and raises the possibility that the sympathetic control of the iris may be modified by endogenous prostaglandins. The significance of the loss of efficacy of prostaglandin E_1 at a concentration of 60 ng/ml in the perfusing medium is not yet clear.

Figure 1

Tritium counts per minute versus time in efflux from the unstimulated iris. Each point is the mean $\pm$ S.E.M. of collected one minute samples of the effluent from 3 irides.

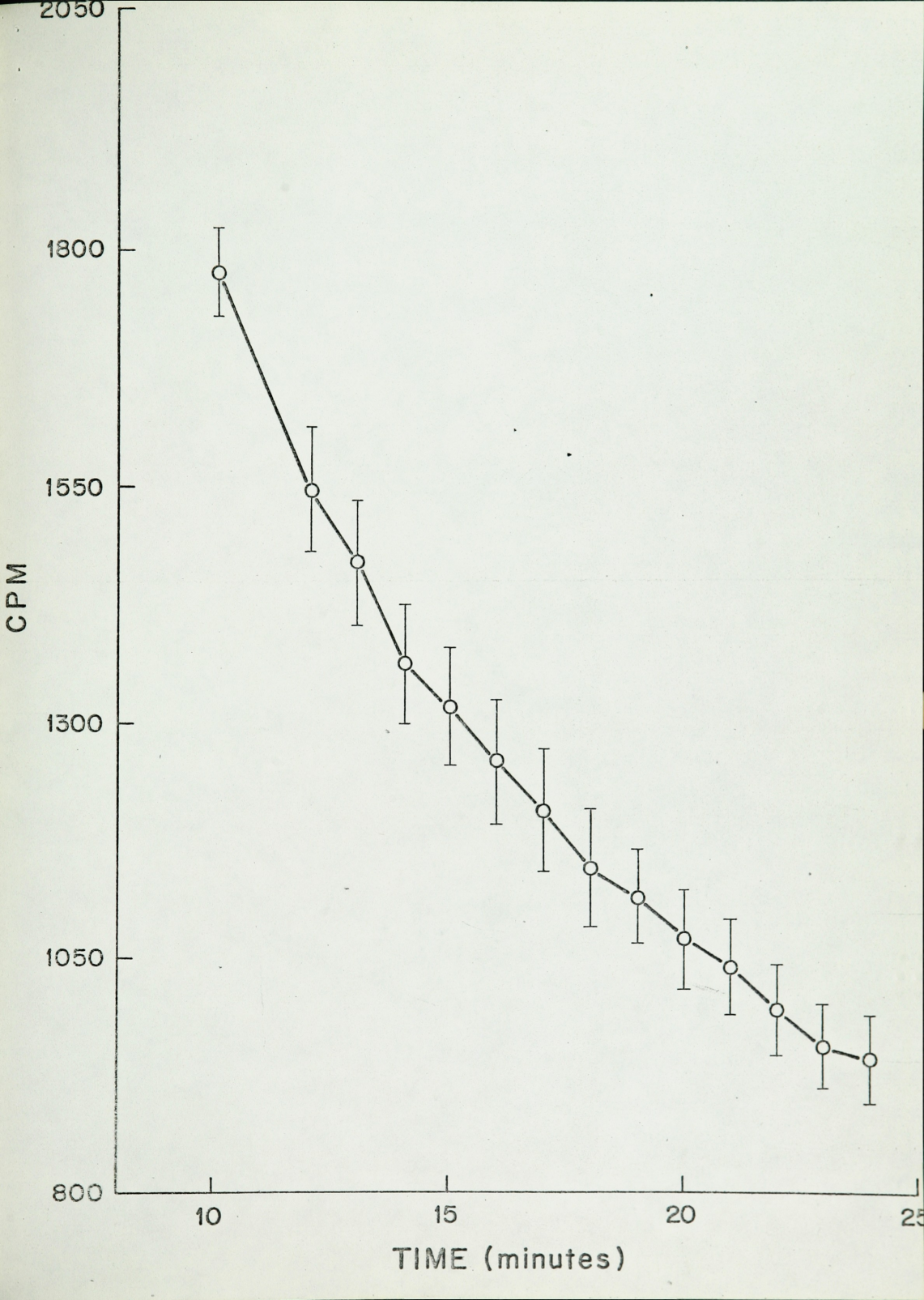

2050
1800
1550
1300
1050
800
CPM
10
15
20
25
TIME (minutes)

Figure 2

Tritium counts per minute versus time in efflux from the electrically field-stimulated iris. Each point is the mean ± S.E.M. of collected one minute samples of the effluent from 6 irides.

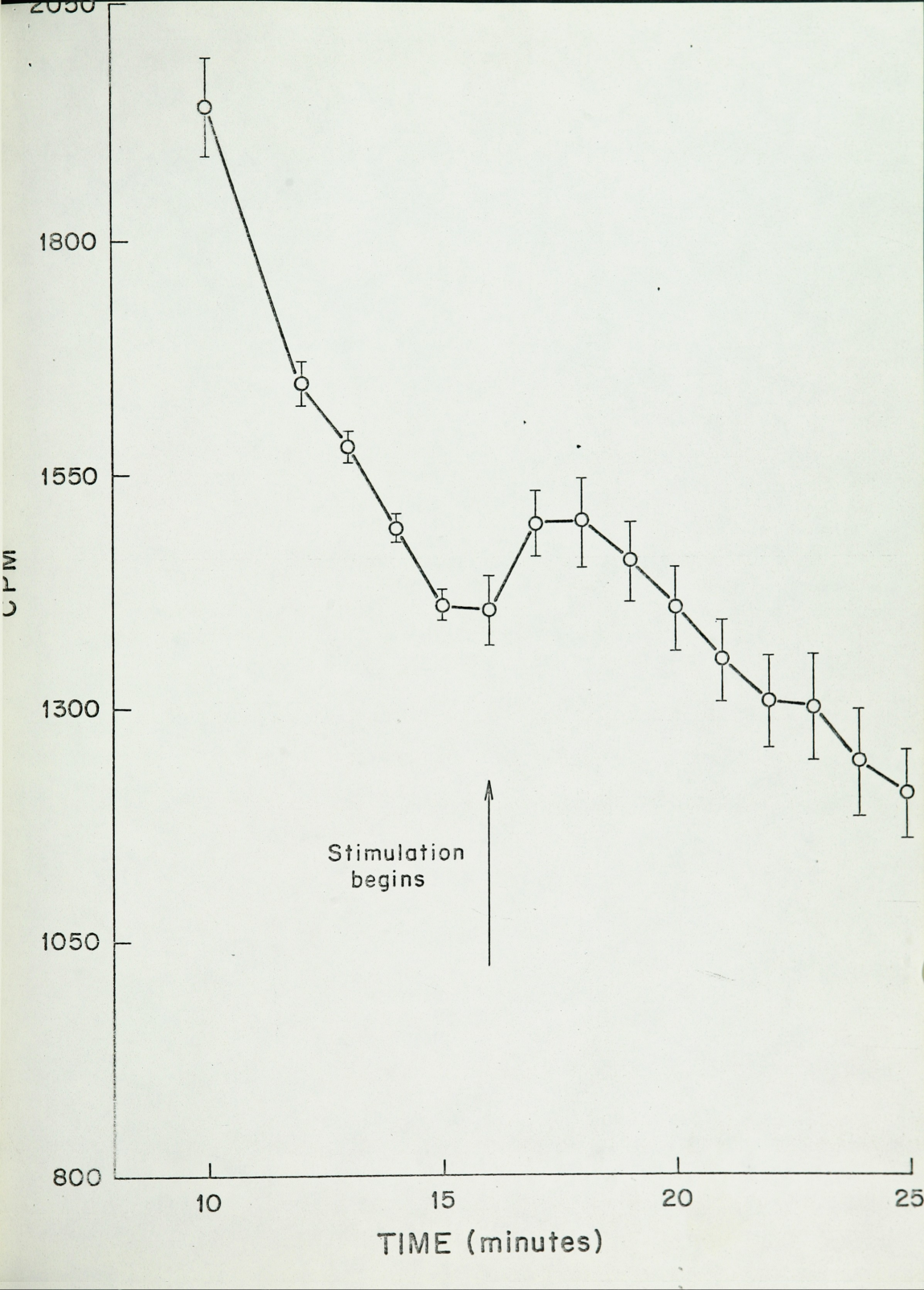

2050
1800
1550
1300
1050
800
CPM
Stimulation
begins
10
15
20
25
TIME (minutes)

Figure 3

Tritium counts per minute versus time in efflux from the electrically field-stimulated iris which has been incubated in ^{3}H-norepinephrine in the presence of 3×10^{-6} M cocaine. Each point is the mean $\pm$ S.E.M. of collected one minute samples of the effluent from 4 irides.

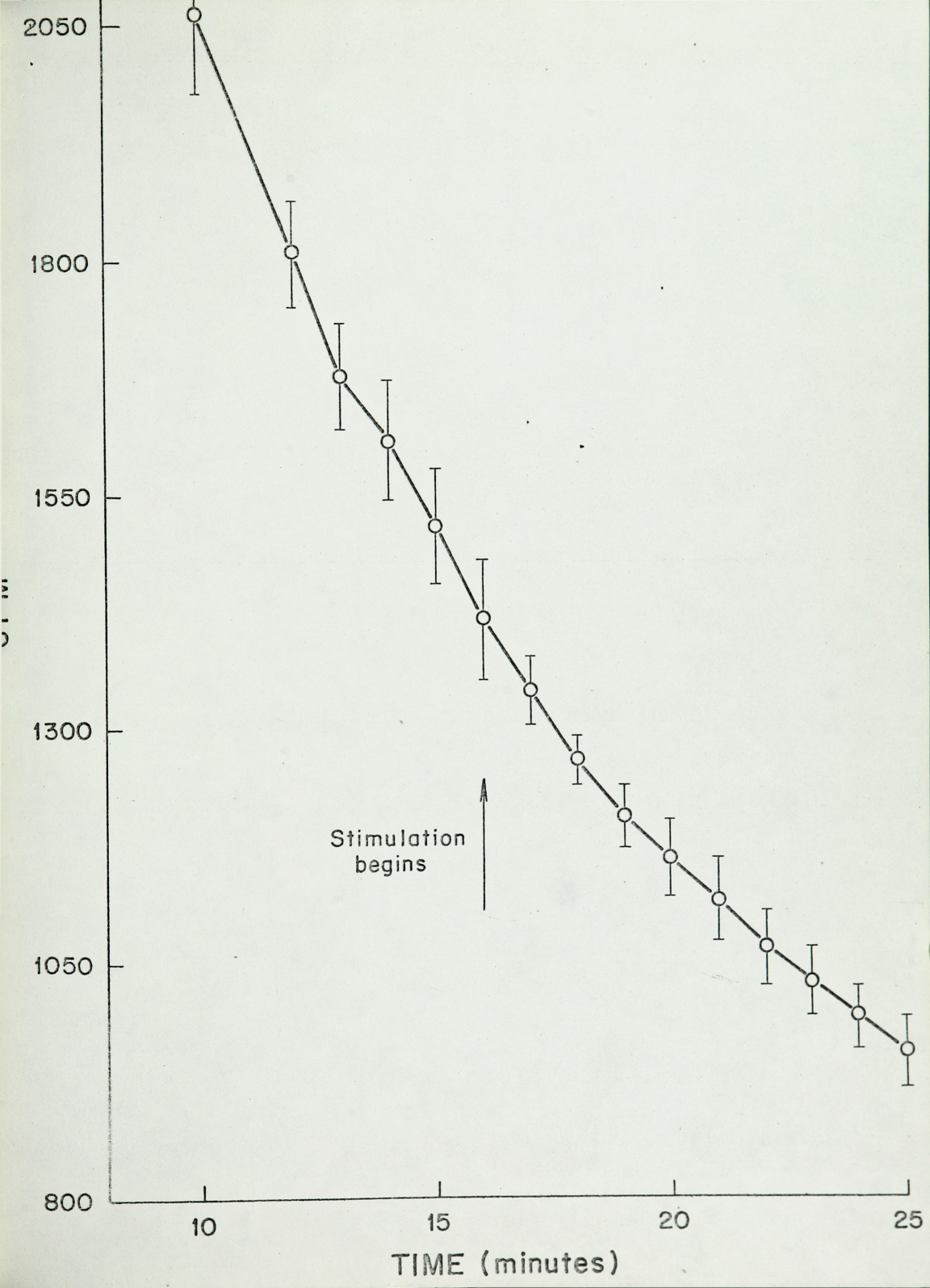

2050
1800
1550
1300
1050
800
Stimulation
begins
10
15
20
25
TIME (minutes)

Figure 4

Tritium counts per minute versus time in efflux from the electrically field-stimulated iris with 10 ng/ml PGE_1 present in the perfusing solution. Each point is the mean ± S.E.M. of collected one minute samples of the effluent from 4 irides.

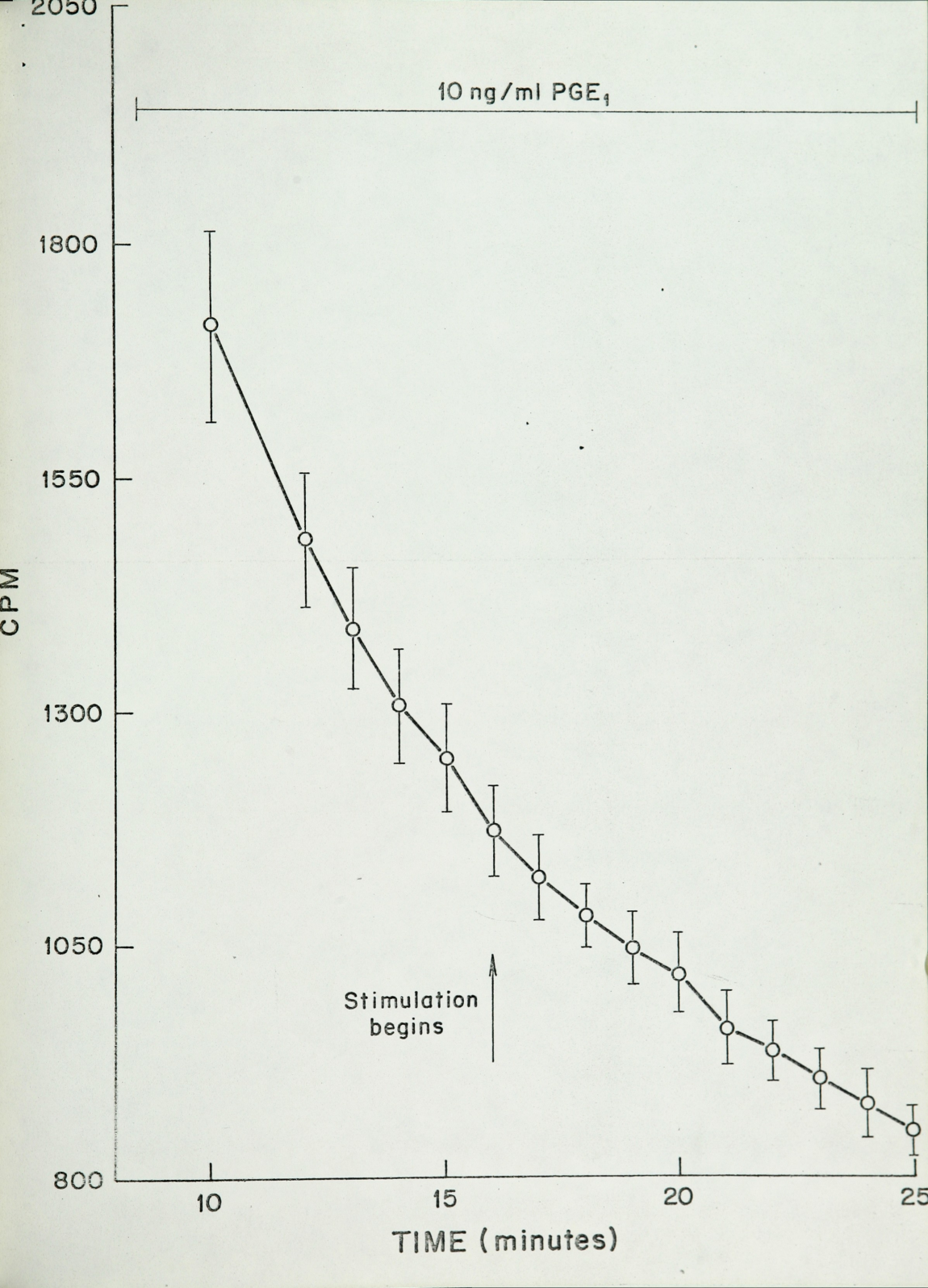
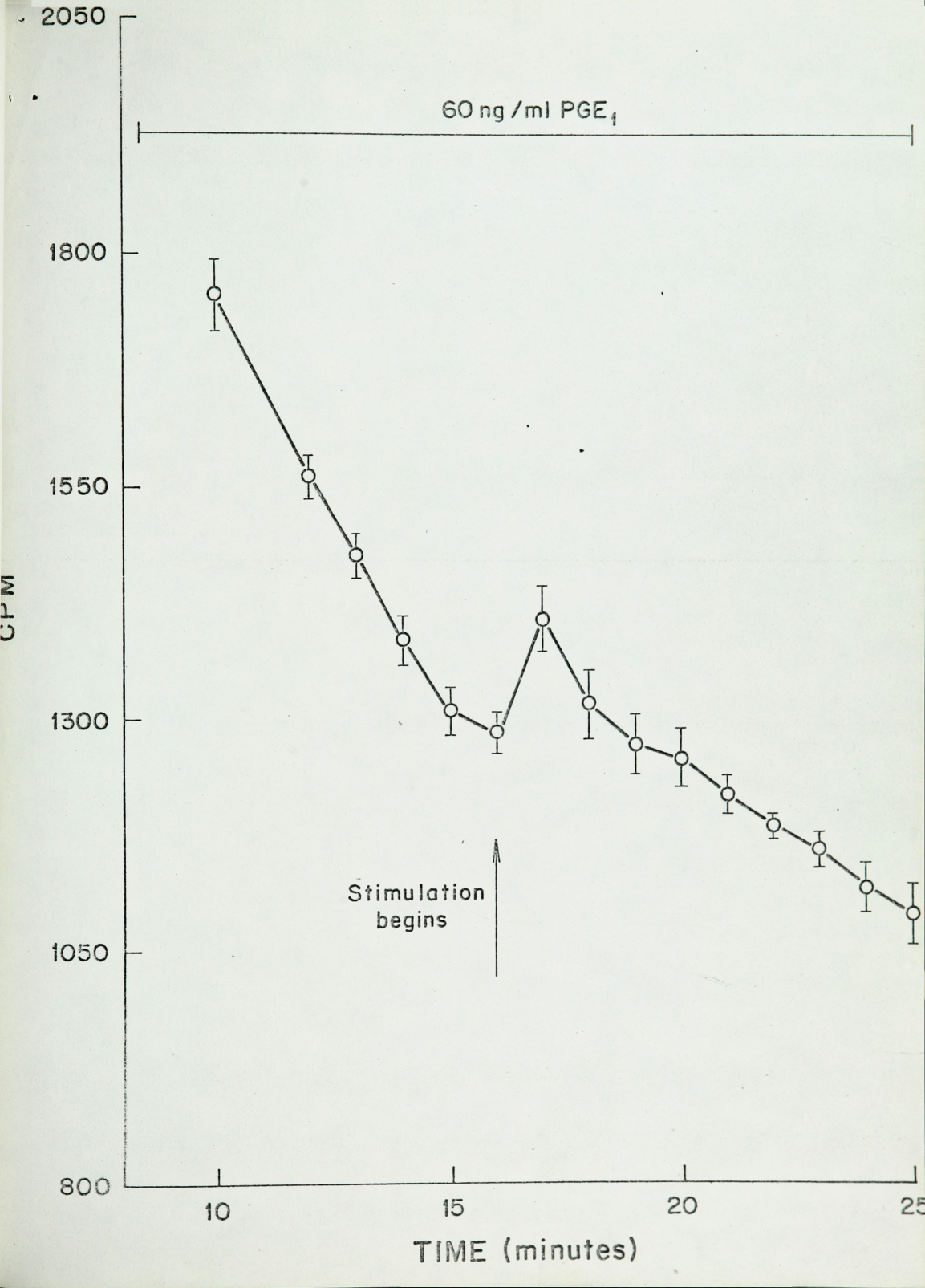

2050
10 ng/ml PGE1
1800
1550
CPM
1300
1050
Stimulation
begins
800
10
15
20
25
TIME (minutes)

Figure 5

Tritium counts per minute versus time in efflux from the electrically field-stimulated iris with 60 ng/ml PGE_1 present in the perfusing solution. Each point is the mean $\pm$ S.E.M. of collected one minute samples of the effluent from 4 irides.


2050
60 ng/ml PGE1
1800
1550
CPM
1300
Stimulation
begins
1050
800
10
15
20
25
TIME (minutes)

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