Short communications

Frequency dependence of dual negative feedback control of secretion of sympathetic neurotransmitter in guinea-pig vas deferens

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The secretion of sympathetic neurotransmitter in guinea-pig vas deferens appears to be normally controlled by two different mechanisms, one dependent and one independent of prostaglandin E. The efficiency of both control systems, as well as of exogenous prostaglandin E_2, is inversely related to nerve stimulation frequency.

Experiments with guinea-pig isolated superfused field stimulated vas deferens indicate that the secretion of noradrenaline induced by nerve stimulation in this preparation is controlled by two different mechanisms, one dependent and one independent of the formation and release of endogenous prostaglandin E (Stjärne, 1973a).

The present work reports experiments showing that the efficiency of both control mechanisms, as well as the effect of exogenous prostaglandin E_2, is inversely related to frequency of nerve stimulation.

Methods.—The experiments were carried out on 30 vas deferentia from 15 guinea-pigs weighing 300–350 g as described in more detail elsewhere (Stjärne, 1973a). The tissue was preincubated in 1 ml Tyrode solution with 10 μCi (260 ng) of [^{3}H]-(−)-noradrenaline (New England Nuclear Corp.) Intact [^{3}H]-noradrenaline was found to account for more than 90% of the total ^{3}H retained in the preincubated tissue, after extensive washing. Desipramine 0·6 μM and normetanephrine 10 μM were present in the superfusion medium to block rebinding of released noradrenaline. In view of this it is assumed that the fractional rise in efflux of total ^{3}H ((evoked rise in efflux of ^{3}H)/(total ^{3}H in the tissue at the time of stimulation)) evoked either by supramaximal field stimulation with 150-600 pulses, 1·5 ms in duration and at frequencies of 1, 5, 10 or 30 Hz in random order, or by raising the K^+ concentration of the medium to 100 mM, gives a valid measure of the secretion of [^{3}H]-noradrenaline. This is further supported by the fact that tetrodotoxin 0·5 μg/ml, which in itself did not affect resting efflux of ^{3}H, completely abolished the rise in effiux of ^{3}H induced by nerve stimulation.

Results.—All results obtained with 300 pulses of nerve stimulation at each frequency, or with high K^+, are represented in Figure 1. When no additions were made, except of desipramine and normetanephrine to block rebinding of released noradrenaline, the average fractional secretion of [^{3}H]-noradrenaline per nerve stimulus increased with stimulation frequency from 1 to 30 Hz (left panel, control). Preincubation for 10 min with 5, 8, 11, 14-eicosatetraynoic acid (ETA) 30 μM to block the formation of prostaglandin E (Downing, Ahern & Bachta, 1970) markedly enhanced the secretion of [^{3}H]-noradrenaline at 1–10 Hz, but not at 30 Hz (left panel, ETA); it did not significantly alter the secretion evoked by high K^+ (upper right panel, $K^+_{ETA}/K^+_{CONTROL}$). When the secretion of [^{3}H]-noradrenaline was expressed in relative terms, taking the secretion at 30 Hz in each individual experiment as 100% (lower right panel) the enhancing effect of ETA treatment was found to be highly significant ($P<0.001$) on stimulation at 1–10 Hz, and inversely related to stimulation frequency (upper right panel, ETA/control). Exogenous prostaglandin E_2 13 nM depressed [^{3}H]-noradrenaline secretion at 1–30 Hz (left panel, ETA + PGE_2); this effect was also inversely related to stimulation frequency (upper right panel, ETA + PGE_2/ETA). The secretion evoked by high K^+ was depressed by exogenous prostaglandin E_2 to about the same extent as was secretion induced by nerve stimulation at 1 Hz (upper right panel, $(K^+_{ETA+PGE2}/K^+_{ETA})$.

Phentolamine (PA) 0·75 μM, added to block α-adrenoceptor functions in the ETA pretreated preparation, very strongly enhanced the secretion of [^{3}H]-noradrenaline induced by nerve stimulation at 1–30 Hz (left panel, ETA + PA); this effect was also inversely related to the frequency of nerve stimulation (upper right panel,

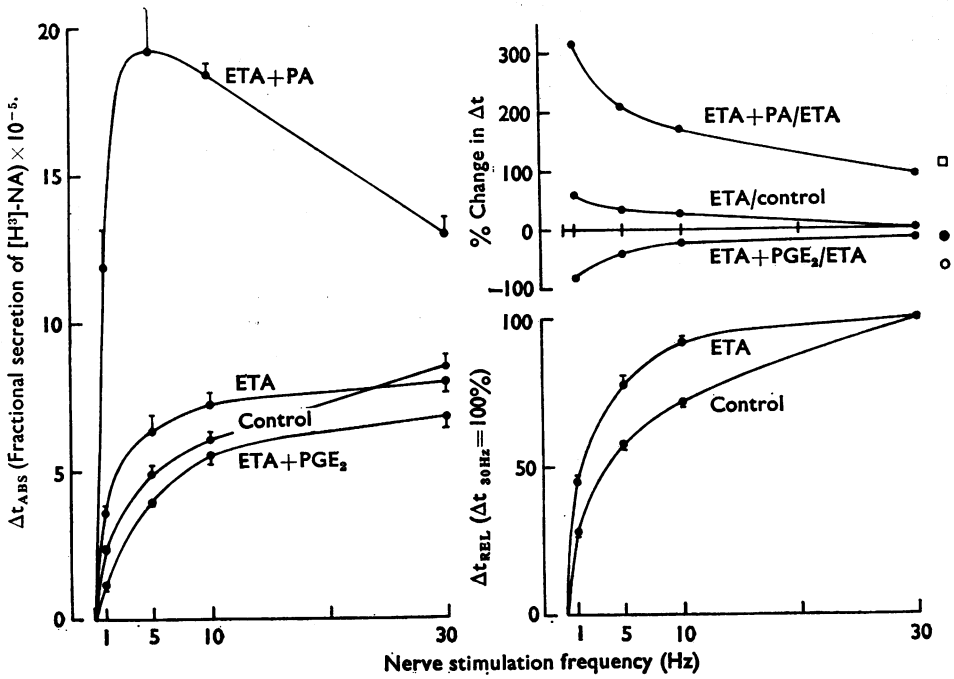

FIG. 1. Left panel: Average fractional secretion of [^{3}H]-noradrenaline ([^{3}H]-NA) per stimulus (absolute$\triangle$t) induced by trains of 300 supra-maximal pulses at 1–30 Hz. ETA=5, 8, 11, 14-eicosatetraynoic acid ; PA=phentolamine ; PGE_2=prostaglandin E_2. Lower right panel: $\triangle$t at 1, 5 and 10 Hz expressed in relative terms, taking$\triangle$t at 30 Hz as 100%. Upper right panel: Relative drug-induced changes in $\triangle$t, and the secretion of [^{3}H]-noradrenaline evoked by high K^+ (100 mM). □=$K^+_{ETA+\ PA}/K^+_{ETA}$; ●=$K^+_{ETA}/K^+_{CONTROL}$; ○=$K^+_{ETA+\ PGE2}/K^+_{ETA}$. Each point represents the average of 4–19 observations. Vertical bars give S.E. For further explanations see Text.

ETA+PA/ETA). In this case the secretion evoked by high K^+ was also enhanced, to about the same extent as that induced by nerve stimulation at 30 Hz, but considerably less than on stimulation at lower frequencies. The absolute fractional secretion of [^{3}H]-noradrenaline when a total of 150 pulses was applied at each frequency, in the presence of phentolamine, was closely similar to that obtained with 300 pulses. On the other hand, the average fractional [^{3}H]-noradrenaline secretion per stimulus, when trains of 600 pulses were applied, was markedly lower, when compared to the levels at 150–300 pulse trains.

Discussion.—The observed frequency/output curve in the presence of desipramine and normetanephrine shows that in guinea-pig vas deferens the average fractional secretion of [^{3}H]-noradrenaline per nerve stimulus, and possibly of total endogenous noradrenaline as well (Hughes, 1973), is not constant but inversely related to the time interval between successive stimuli. This shows that the secretory mechanism is sensitive to facilitation/summation processes (Stjärne, 1970), especially on stimulation at low frequency where the slope of the curve is particularly steep. Assuming that ETA selectively acts by completely blocking prostaglandin E formation, and phentolamine by blocking α-adrenoceptors, the present results support previous evidence that nerve stimulation induced secretion of noradrenaline in guinea-pig vas deferens is normally restricted by two different control mechanisms, one dependent and one independent of prostaglandin E (Stjärne, 1973c). As might be expected the efficiency of both mechanisms, as well as of exogenous prostaglandin E_2, was found to be inversely related to the stimulation frequency (Junstad & Wennmalm, 1973 ; Stjärne, 1973a). The fact that local depolarization with high K^+,

in preparations preincubated with ETA, was enhanced by α-adrenoceptor blockade indicates that the prostaglandin E-independent control mechanism does not operate to any major extent by regulating invasion of the nerve terminals by propagated nerve impulses, but by restricting secretion in individual secretory junctions (Stjärne, 1973b). Since secretion of [^{3}H]-noradrenaline induced by high K^+ was strongly inhibited by exogenous prostaglandin E_2, the failure of ETA to enhance K^+-induced secretion suggests that this type of stimulus did not successfully trigger the local formation and release of prostaglandin E (Stjärne, 1973b).

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