The bioassay technique depends on transferring the Krebs solution bathing the donor tissue to a cascade system where the active material is assayed on a series of superfused vascular tissues. This technique allows the detection of as little as 50 pg of noradrenaline added to the 4 ml donor bath. The rabbit portal vein (Hughes & Vane, 1967) and the rabbit vas deferens were used as donor tissues and the intramural nerves excited by transmural stimulation (1 ms, supramaximal voltage at 1–16 Hz for 120, 240 and 480 pulses).

The release of noradrenaline-like material was seen at all frequencies, and was abolished in the presence of bretylium (10^{-6}–10^{-5} g/ml) or tetrodotoxin (5×10^{-7} g/ml). The contractions of the assay vessels to noradrenaline and to the released material were also abolished by phentolamine (10^{-9}–10^{-8} g/ml). Further proof that the active material was noradrenaline was obtained by combining the relatively large outputs from the vas, concentrating the samples over alumina, and estimating the noradrenaline fluorimetrically.

The most obvious feature of the outputs from the vein and vas deferens was the much greater output per pulse seen when the frequency was increased from 1–4 Hz to 8 Hz and above. Thus at 2 Hz the mean output from the vein was $11{\cdot}9\pm0{\cdot}86$ (pg/pulse)/g (mean$\pm$S.E.M.); this increased to $30{\cdot}7\pm3{\cdot}97$ (pg/pulse)/g at 8 Hz (ten experiments each). This difference was significant ($P<0{\cdot}01$), and was not altered by treating the tissues with cocaine. In the vein and the vas deferens, cocaine (5 μg/ml) increased the noradrenaline output by 200–300% at both low (1–4 Hz) and high (8–16 Hz) frequencies of stimulation.

These experiments indicate that the neuronal uptake mechanism for noradrenaline is as active at high as at low frequencies of stimulation. The differences in output per pulse contrast with results obtained in the spleen. Metabolism of the noradrenaline may contribute to this difference in measured outputs (Langer, 1970) and this and other possible mechanisms are being investigated.

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Motor transmission in the vas deferens: the inhibitory action of noradrenaline

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It is generally held that the post-ganglionic motor transmitter in the guinea-pig vas deferens is noradrenaline. The following findings do not support this view:

1. In Krebs-Henseleit solution at 35° C it was often impossible to mimic with noradrenaline the tetrodotoxin-sensitive twitch elicited by field stimulation with 3–10 pulses (1 ms; 10 Hz; supramaximal voltage). Some preparations were very insensitive to the motor action of noradrenaline, 125 μg/ml failing to match twitches; even in sensitive preparations large doses, 10–25 μg/ml, were needed for such a match.

2. In lower doses (1–5 μg/ml) noradrenaline always inhibited the twitches (Fig. 1B), sometimes totally. This is not due to excitation of β-adrenoceptors, since the β-adrenoceptor blocking agents, propranolol and pronethalol (1–2 μg/ml), did not antagonize and even accentuated this noradrenaline-inhibition (D), although antagonizing isoprenaline-inhibition (compare C with A). On the other hand, the noradrenaline-inhibition was partially antagonized (F) by the α-adrenoceptor blocking agent phentolamine (1–10 μg/ml). Furthermore, contractions induced by 5-methylfurmethide were depressed by isoprenaline but not by noradrenaline (1–5 μg/ml); therefore noradrenaline did not exert a relaxant effect on the muscle.

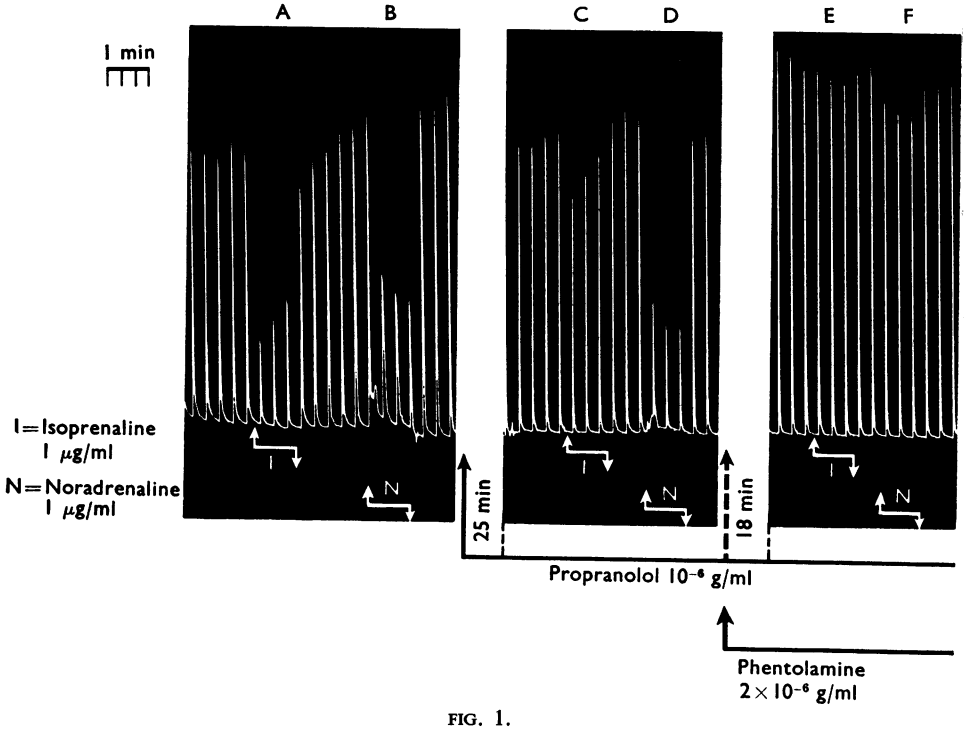

FIG. 1.

3. Twitches were unaffected by: (*a*) α-blockade with phenoxybenzamine (1 μg/ml), even though the twitch-matching dose of noradrenaline increased 1,000×; (*b*) phentolamine; (*c*) α+β blockade with phentolamine/phenoxybenzamine+propranolol; (*d*) reserpinization; (*e*) hexamethonium; (*f*) atropine; (*g*) eserine.

4. Tyramine or amphetamine (1 μg/ml), both known to promote noradrenaline release from nerve-endings, drastically inhibited twitches but potentiated noradrenaline contractions.

5. The monoamine oxidase inhibitor, tranylcypromine (1 μg/ml), considerably potentiated noradrenaline contractions but depressed twitches.

There is evidence for a high noradrenaline content in the vas. The above findings make it necessary to reconsider the role of noradrenaline in this tissue; besides vasomotor control, its function here might be to inhibit the motor transmission.

Similar results with phenoxybenzamine were obtained in rabbit vas deferens.

Adrenaline uptake mechanisms in the rat uterus

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The motor response to adrenaline in the presence of isoprenaline in the pregnant and oestradiol-treated uterus is blocked by desipramine and other uptake-blocking agents (Tothill, unpublished). No motor response occurs in the ovariectomized or dioestrous rat uterus. As this response to adrenaline may therefore be associated in some way with uptake mechanisms these were investigated in the uteri of late pregnant and ovariectomized rats.

Sprague Dawley rats weighing about 200 g were ovariectomized and left for 3 weeks before the experiment. Pregnant animals were used on the twentieth day after mating. Animals were killed by a blow on the neck and the uterus was removed. Strips of the pregnant uterus weighing about 50 mg and horns of the uteri from ovariectomized rats weighing about 40 mg were incubated at 37° C for 30 min in mammalian Ringer solution with ^{14}C-adrenaline. After 30 min the tissue was removed, blotted and weighed. The tissue was placed in vials containing 1 ml of distilled water with 1 ml M hyamine hydroxide in methanol, hermetically sealed and placed in an oven at 60° C for 3 h. The resultant clear solution was counted in a liquid scintillation counter.

Adrenaline uptake was studied at concentrations ranging from 5 ng/ml to 20 μg/ml. In uteri from ovariectomized rats the uptake per gramme of tissue was never more than twice the concentration in the incubation fluid expressed as ng/ml, but in pregnant uteri this was usually less than half. The greatest difference of uptake between these two hormonal states occurred at low concentrations of adrenaline (5–100 ng/ml). Desipramine (1 μg/ml), an inhibitor of uptake 1 (Iversen, 1965), reduced the uptake of adrenaline (100 ng/ml) by 23·6% ($P<0.025$) in uteri from ovariectomized rats but had no effect in the uteri from pregnant rats.

Desipramine reduced the uptake of adrenaline (5 ng–5 μg/ml), but not above 5 μg/ml, in the ovariectomized rat uterus. Metanephrine (20 μg/ml), an inhibitor of uptake 2 (Burgen & Iversen, 1965), reduced by 5·6% the uptake of adrenaline in the uteri of ovariectomized rats at concentrations of 10 μg/ml ($P<0.5$) but had no effect on uptake into uteri from pregnant animals. The uptake of adrenaline at low concentrations which could be blocked with desipramine was probably accounted for by uptake into sympathetic nerves, since they are usually associated with uptake 1. During late pregnancy the uterus has increased in bulk by about 20 times, so that neuronal uptake per gramme of tissue might not be detectable. It seems possible that there may be only one main uptake mechanism in the rat uterus and its relation to the motor response is not clear.

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