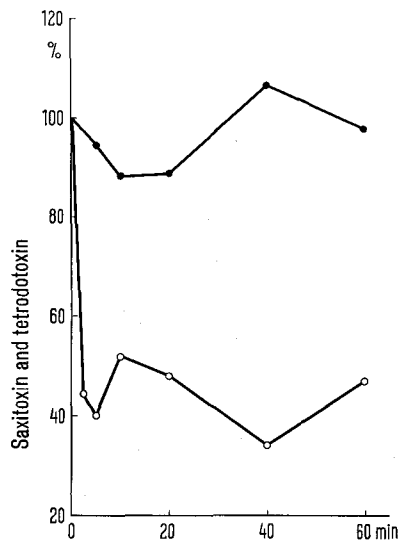
chloride formed when the samples were brought to pH 2.5–3.5. Salt tends to prolong the death times in this assay[15,18,19]. Smaller doses of poison result in longer death times, so the toxicity remaining after heating could be estimated from published tables[17] or from graphs of the dose/death time previously determined by us. Doses of less than 1 MU cannot be assayed by this technique, therefore the percentage of poison that remained after heating could be measured only if it was not less than 25% of the initial concentration.

Virtually every sample of STX showed better stability than the corresponding sample of TTX, heated at the same pH for the same length of time. In strongly acid solutions TTX rapidly lost toxicity at 100°C, while STX remained stable, but above pH 2 there was less difference. The Figure shows the results of heating the poisons at pH 1.0 for times up to 60 min. There was no significant change in the toxicity of the STX even after 60 min at 100°C, but the TTX lost more than half its toxicity within the first few minutes. At pH 0.64 TTX lost toxicity even more rapidly, while STX was as stable at pH 0.64 as at 1.0.



Amounts of saxitoxin (●) and tetrodotoxin (○) found remaining in solutions which had been heated to 100°C for 2.5–60 min at pH 1.0, expressed as a percentage of the amount of poison initially present.

STX is not indefinitely stable when heated with concentrated HCL[20,21], but in 3N acid there is only a slight loss of toxicity after 24 h at 100°C[10]. One would not expect significant loss of toxicity after heating for 1 h or less at pH 1.0 and the results reported here have confirmed this. On the other hand, they show that TTX loses activity rapidly at pH 1.0 and 100°C, and can easily be distinguished from STX by this property. In more strongly acid solutions the destruction of TTX is even greater, but difficulties might arise if weakly toxic solutions were tested below pH 1.0 because of the larger amounts of salt that would be formed when the more concentrated acid was neutralized before bioassay. The effect that salt has in prolonging survival times in this assay makes the estimation of near-threshold amounts of poison unreliable or even impossible in concentrations of NaCl greater than about 0.1 *M*[15,18,19].

It is therefore suggested that bio-assay of suitable dilutions, before and after heating to 100°C at pH 1.0 for 20–30 min, is a simple and reliable way for distinguishing between solutions of TTX and STX.

Resumen. La tetrodotoxina pierde toxicidad rapidamente cuando se calienta en solución acida por debajo de pH 2, mientras que la saxitoxina es estable. Se puede distinguir facilmente entre estos venenos calentándolos a 100°C en solution pH 1.0 durante 20–30 min.

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Evidence for a Different Site of Action of Phenoxybenzamine and Desmethylimipramine on the Catecholamine Uptake System

It is well known that both phenoxybenzamine and desmethylimipramine behave as very potent blockers of the catecholamine uptake system (AXELROD et al.[1]; SIGGS et al.[2]; FURCHGOTT and KIRPEKAR[3]; TITUS et al.[4]; IVERSEN and LANGER[5]). The blocking effect induced by these agents could be considered essentially irreversible.

The purpose of the present experiments was to determine whether the site of action of phenoxybenzamine and desmethylimipramine, on adrenergic nerve terminals, is or is not the same. The changes in sensitivity of an atrium to norepinephrine was used as indication of the catecholamine uptake blockade induced by either phenoxybenzamine or desmethylimipramine in the presence or in the absence of a protecting agent (FURCHGOTT[6].) Provided that norepinephrine and tyramine are taken up by a common uptake system (COMMARATO et al.[7]; FURCHGOTT et al.[8]; SÁNCHEZ GARCIA et al.[9]), tyramine was used in these experiments in order to protect the uptake system for catecholamines, during exposure of atria to the blocking agents.

Methods. Guinea-pigs weighing from 300–500 g were used. The animals were killed by a blow on the head. The hearts were removed and the left atria were prepared for mounting as previously described by FURCHGOTT and SÁNCHEZ GARCIA[10]. In each experiment one half of the atrium served as a control. The bathing solution was

Krebs-bicarbonate containing 10 mM glucose and 10^{-5} g/ml of ethylene diamine tetraacetic acid (EDTA). A mixture of 95% oxygen and 5% carbon dioxide was bubbled through the solution. Atria were attached to force-displacement transducers (Grass, mod. FT.03) and subjected to electrical stimulation at a frequency of 30 beats/min. Mechanical activity was recorded by means of a Grass Polygraph. Each atrium was subjected to a resting tension of 1 g. The animals were given reserpine (5 mg/kg) i.p. 18 to 24 h prior to the experiments.

Sensitivity of an atrium to norepinephrine was evaluated by increasing the concentration of the drug in a stepwise manner (3-fold increase by step) until a concentration was reached which produced maximal response. Responses are expressed as changes in per cent of maximal amplitude of contraction. Tyramine, when used as a protecting agent, was present in the chamber 5 min before and during the exposure to both phenoxybenzamine and desmethylimipramine.

The doses of drugs used (norepinephrine bitartrate, tyramine hydrochloride, phenoxybenzamine hydrochloride and desmethylimipramine hydrochloride) are expressed in terms of g of salts per ml of medium in the muscle chamber.

Results. All experiments reported in this paper were carried out with reserpinized preparations in order to avoid the interference of the norepinephrine releasing effect of tyramine and phenoxybenzamine in the evaluation of results. After a 30 min equilibration period, all preparations were initially tested for sensitivity to norepinephrine. Two dose-response curves were usually determined before the atria were exposed to the blocking agents.

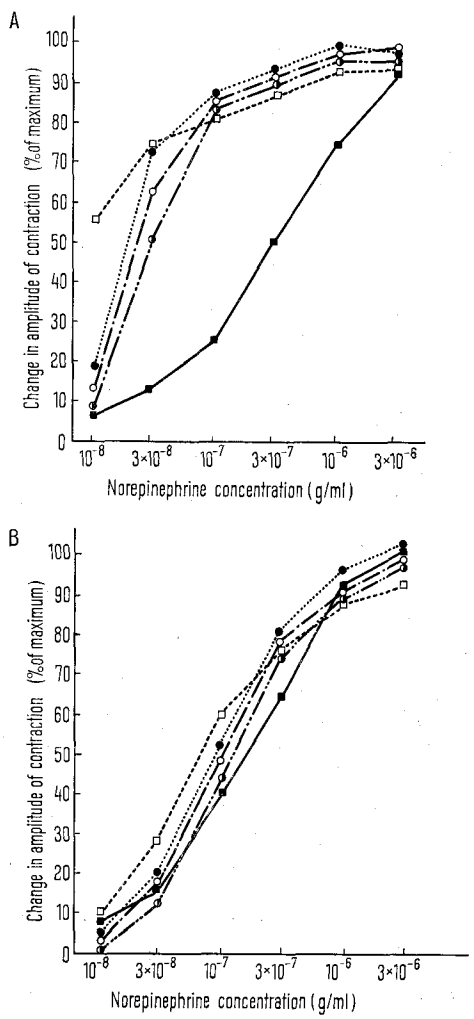

Fig. 1. Ability of tyramine to protect against irreversible potentiation, of the norepinephrine (NE) responses, induced by phenoxybenzamine (PBZ). A) Control half. ■—■, responses to cumulative doses of NE before treatment. 10 min later the preparation was exposed to phenoxybenzamine (10^{-6}, for 20 min) and dose-response curves for NE were repeated 20 min (□---□); 50 min (●···●); 80 min (○-·-·-○); and 110 min (◑-·-·-◑) later. B) Experimental half. ■—■, responses to cumulative doses of NE before treatment. 10 min later the preparation was exposed to tyramine (10^{-4}, for 5 min) and without previous washing to PBZ (10^{-6}, for 20 min). Dose response curves for NE were repeated 20 min (□---□); 50 min (●···●); 80 min (○-·-·-○); and 110 min (◑-·-·-◑) later. The dose-response curves shown in A and B are from two halves of single left atrium.

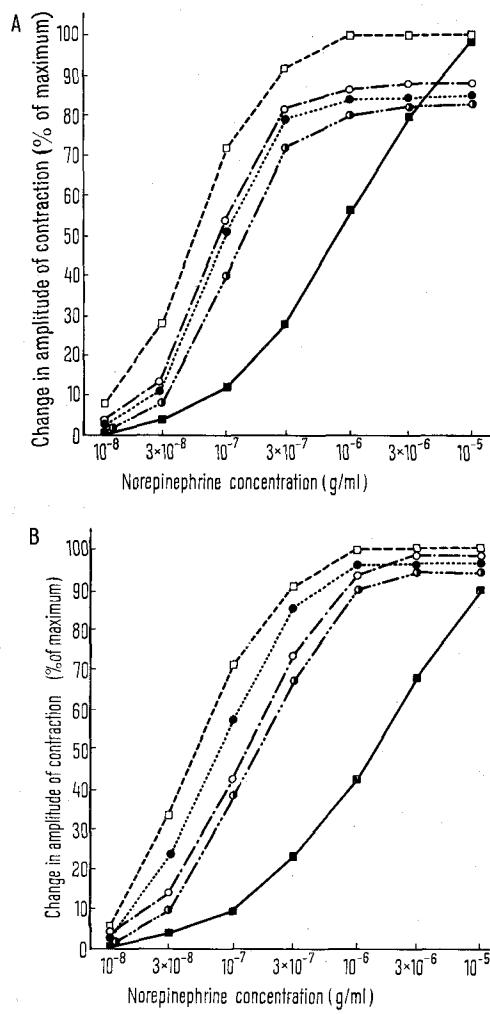

Fig. 2. Lack of ability of tyramine to protect against irreversible potentiation, of the norepinephrine (NE) responses, induced by desmethylimipramine (DMI). A) Control half. ■—■, responses to cumulative doses of NE before treatment. 10 min later the preparation was exposed to DMI (10^{-6}, for 20 min) and dose-response curves for NE were repeated 20 min (□---□); 50 min (●···●); 80 min (○-·-·-○); and 110 min (◑-·-·-◑) later. B) Experimental half. ■—■, responses to cumulative doses of NE before treatment. 10 min later the preparation was exposed to tyramine (10^{-4}, for 5 min) and without previous washing to DMI (10^{-6}, for 20 min). Dose-response curves for NE were repeated 20 min (□---□); 50 min (●···●); 80 min (○-·-·-○); and 110 min (◑-·-·-◑) later. The dose -response curves shown in A and B are from two halves of single left atrium.

In a group of 7 experiments, 10 min after the last dose-response curve for norepinephrine, one half of the atrium received a dose of tyramine (10^{-4}) and 5 min later (without previous washing) both were exposed to phenoxybenzamine (10^{-6}, for 20 min). Dose-response curves for norepinephrine were repeated 20, 50, 80 and 110 min later in both halves. Figure 1 illustrates the results of a typical experiment. It can be seen that the irreversible increase in sensitivity to norepinephrine (Figure 1-A) which appears after phenoxybenzamine treatment, is inhibited (Figure 1-B) when the atrium was first exposed to tyramine. In another set of 8 similar experiments desmethylimipramine was used instead of phenoxybenzamine. After initial dose-response curves for norepinephrine were determined, one half of the atrium received tyramine (10^{-4}) and 5 min later both were exposed to desmethylimipramine (10^{-6}, for 20 min). Sensitivity to norepinephrine was again tested 20, 50, 80 and 110 min later. Figure 2 shows the results of a typical experiment. It is interesting to observe that, in this particular case, no difference in sensitivity to norepinephrine was found between the control (Figure 2 A) and tyramine-treated preparation (Figure 2 B), when they were exposed to desmethylimipramine.

Discussion. It is well established that phenoxybenzamine and desmethylimipramine potentiate the responses to norepinephrine. This potentiating effect is believed to be due to the ability of both, phenoxybenzamine and desmethylimipramine, to inhibit the uptake of norepinephrine into the nerve terminals.

The results of the present investigation show that the potentiation of the responses to norepinephrine induced by phenoxybenzamine, can be prevented by the presence of tyramine during the period of exposure to phenoxybenzamine. These findings provides further evidence for a common uptake site for norepinephrine and tyramine on adrenergic nerve terminals. On the other hand, our results show that tyramine, when present during exposure to desmethylimipramine, was not capable of preventing the potentiation of norepinephrine responses induced by this blocking agent.

Provided that both, phenoxybenzamine and desmethylimipramine, share the capacity to block the uptake of norepinephrine by adrenergic neurones, the fact that tyramine was able to prevent the uptake blocking action of phenoxybenzamine but not that of desmethylimipramine, can be taken as an evidence for a different site of action on catecholamine uptake system.

Resumen. El tratamiento con fenoxibenzamina o desmetilimipramina da lugar a una potenciación irreversible de las respuestas a noradrenalina, en la aurícula aislada de cobayo. La presencia de tiramina durante el tratamiento previene dicha potenciación cuando se utiliza fenoxibenzamina, mientras que no la modifica en el caso de la desmetilimipramina.

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Effect of Constant Lighting on the Morphine Susceptibility Rhythm

Morphine sulfate displays quantitative variations of susceptibility along a 24-hour time scale characterized by peak analgesia (i.e. crest) during the dark and a trough in the light period[1]. Reversal of the lighting regimen produced an inversion of the morphine susceptibility pattern suggesting a prevalent photoperiodic effect on morphine susceptibility[2]. Investigators have reported that pentobarbitol susceptibility was suppressed by programming mice in continuous darkness[3]. Similarly, pineal epinephrine rhythms have been abolished in rats maintained in continuous darkness or blinded by bilateral orbital enucleation[4]. Some 24-hour rhythms have been suppressed or abolished by constant lighting[5,6]. A continuous lighting schedule effect is reported herein as further evidence of the dominant role of photoperiodicity on the susceptibility pattern of morphine analgesia.

Adult female albino CF-1 mice (25.4 ± 0.4 g body wt.) were housed and tested with conditions, procedures and morphine dosage as previously reported[1,2], except for the constant lighting schedule and incrased body weight of about 2 g due to aging over the 4 week test period. An initial standard light period (I) had lights on 06.05–18.05 h and total darkness from 18.05–06.05 h[1]; a second experiment (II) had the lighting regimen reversed[2] and in this study (III) the mice were exposed to continuous light for a period of 14 days prior to the morphine administration. A standardization time of 14 days is considered adequate to facilitate resynchronization[7].

Beginning at 12.00 h and at 3-hour intervals over the succeeding 24-hour period, groups of 20 mice were pretested, weighed and injected i.p. with morphine sulfate, 8 mg/kg. Exactly 20 min after the morphine injection each mouse was retested for the presence of pain as determined by a modified Haffner tail pinch clamp method[8]. All mice failing to display a positive pain response (i.e. biting clip) within 30 sec were considered to be analgesic[8]. Each analgesic response was converted to per cent analgesia relative to the total number of mice

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