Biochemical Pharmacology, Vol. 27, pp. 1693–1694.

0006-2952/78/0615-1693$02.00/0

SHORT COMMUNICATIONS

Effects of pyrovalerone on peripheral noradrenergic mechanisms

(*Received* 31 *January* 1977; *accepted* 12 *January* 1978)

In the past few years Sayers and Handley [1] proposed a pharmacological classification of stimulating drugs. This classification was based on the suppression of behavioural action of drug after a pretreatment by either α-methyl-*p*-tyrosine (α-MT) or both α-MT and reserpine. In the case of pyrovalerone, its behavioural effects were abolished by the last treatment [1–3]. These results could be explained by a biochemical alteration of the noradrenergic mechanism. The present study was undertaken to investigate the effects of pyrovalerone on the uptake, release and turnover rate of norepinephrine (NE) in rat heart.

MATERIALS AND METHODS

Uptake of [^{14}C]*norepinephrine.* Hearts of male rats (Sprague–Dawley, 200–220 g) were rapidly isolated and perfused, according to Langendorff with Krebs–Henseleit medium containing [1-^{14}C]NE (14.7 ng/ml). 1-[^{14}C]NE (sp. act. 25 mCi/mM) was obtained from Radiochemical Centre, Amersham, U.K. Perfusion conditions (5 ml/min, at 37°C and bubbling of the perfusion medium with a mixture of 95% O_2 and 5% CO_2) were kept constant throughout the experiment. Pyrovalerone was added at various concentrations (2.10^{-4}–10^{-8} M) to the perfusion medium to determine the NE uptake inhibition.

Release of [^{14}C]*norepinephrine.* Isolated hearts were initially perfused, as described above, with the [1-^{14}C]NE perfusion medium for 5 min. Perfusion was then continued for 15 min with pyrovalerone (10^{-6} M) in the [1-^{14}C]NE free medium.

Turn over rate of norepinephrine in rat heart. Tracer doses (20 μCi/kg of [1-^{3}H]NE) obtained chromatographically pure from Radiochemical Centre, Amersham (sp. act. 20 Ci/mM) were injected into the tail vein of rats. Ten min later, the animals were given orally pyrovalerone 5 mg/kg. The rats were killed 1, 2, 3, 4 and 6 hr later. The hearts were removed, and endogenous NE and [^{3}H]NE were measured.

In all the experiments (uptake, release and turnover rate) heart was blotted, weighed and homogenized in 0.4 *N*-perchloric acid containing 0.5% EDTA and 0.5% sodium bisulfite. Catecholamines were isolated according to Anton and Sayre [4]. Endogenous NE was determined by the fluorometric method of Euler and Lishajko [5]. Alumina columns eluates were measured for radioactive NE by liquid scintillation counting. The deaminated metabolites were determined according to Crout [6] and their amounts, less than 5 per cent of total NE, were not taken into consideration for in the results. All data were corrected for respective recovery.

RESULTS AND DISCUSSION

The [^{14}C]NE uptake was 72.0 ± 5.8 ng/g after 5 min of perfusion. This result is in agreement with the values reported by Iversen [7] for NE uptake 1. Pyrovalerone inhibited the NE uptake; the percentage of this inhibition is plotted against the log of pyrovalerone concentration in the medium (Fig. 1). The 50 per cent inhibitory concentration (ID_{50}) was equal to 2.10^{-8} M. Pyrovalerone is a powerful inhibitor of NE uptake, quite similar to imipramine. Much stronger doses of amphetamine 10^{-7} M or other stimulating drugs, must be used in order to obtain the same effects [8].

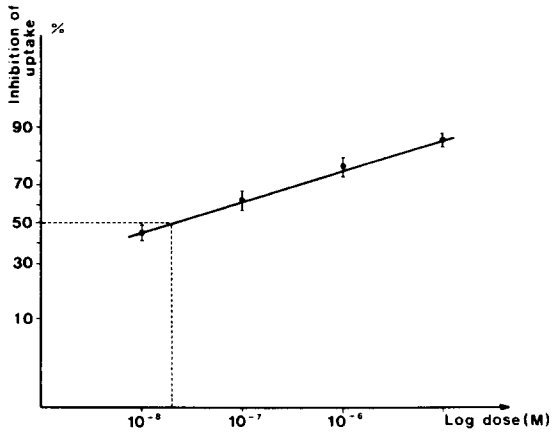

Fig. 1. Percentage of [^{14}C]NE uptake inhibition is plotted against the log of pyrovalerone concentration.

The effects of pyrovalerone on NE release are reported in Table 1. Isolated hearts were first perfused with [^{14}C]NE then washed out with an amine free medium. The rapid wash out of NE from the easily accessible pool, including vascular and extravascular spaces, was followed after 5 min by a slow efflux from adrenergic neurons [9]. Pyrovalerone was then added to the perfusion medium. The ratios of the [^{14}C]NE in the perfusion to the [^{14}C]NE in the heart tissue were measured as an index of norepinephrine release. Pyrovalerone increased the [^{14}C]NE release. No modification was observed on NE sp. act. in heart.

Table 1. NE release in rat heart

	NE efflux index	Sp. act.
Control	3.03 ± 0.20	0.026 ± 0.002
Pyrovalerone 10^{-6} M	*4.34 ± 0.18	0.023 ± 0.002

Heart was perfused with [^{14}C]norepinephrine (14.7 ng/ml) for 15 min and washed with an amine free medium for 5 min. Perfusion was then continued for 15 min with Pyrovalerone. The index of NE efflux was the ratio of [^{14}C]NE in the perfusion medium to the ^{14}C in heart tissue. The sp. act. of heart NE was obtained by dividing the [^{14}C]NE (ng/g) by the endogenous NE (ng/g) in heart. Each value is the mean ± S.M. of six determinations.

* Significantly different from control group, $P < 0.01$.

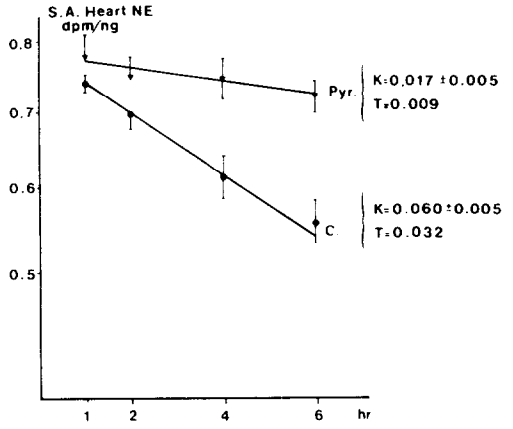

Fig. 2. Specific activity of heart Norepinephrine after an i.v. injection of [^{3}H]NE. Rats were treated 10 min after the NE administration with pyrovalerone (5 mg/kg P.O. ▼—▼ PYR) or saline (0.2 ml/kg P.O. ●—● C). Data is plotted as dpm [^{3}H]NE/ng of endogenous NE vs time in hr. Each dot represents the mean of at least five hearts ± S.M. The slope of decline was plotted by the least squares method. Rate constant of NE loss (K) was expressed in hr^{-1} ± S.M. and turnover rate T was expressed in μg/hr.

These data suggested that pyrovalerone induced a release from both pools of NE (endogenous and [^{14}C]NE). The easily released [^{14}C]NE might be considered as the newly accumulated or newly-synthesized NE [10]. In contrast most stimulating drugs like amphetamine are believed to cause a preferential release of newly synthesized NE [11–13].

The inhibition of NE uptake and the effects exerted on NE release by pyrovalerone altered the noradrenergic transmission. Under our experimental conditions the injection of a tracer dose of [^{3}H]NE determined a mono-exponential disappearance which indicates that [^{3}H]NE is localised in a single pool [14, 15]. The slope of this exponential curve gave a half life of approximately 11.5 hr for NE in the control heart. This result is in good agreement with previous papers [16, 17]. Pyrovalerone decreased the NE turnover rate in rat heart (Fig. 2).

The overall data concerning the biochemical effects exerted by pyrovalerone have revealed a two-impact on the noradrenergic system. On one hand pyrovalerone inhibited the NE uptake and on the other hand released the NE from the storage and functional pools. Furthermore pyrovalerone decreased the NE turnover rate. These biochemical data comply with the basis of the pharmacological classification of Sayers and Handley [1]. The pretreatment by α-MT which inhibited the NE biosynthesis, abolished the amphetamine effects. In the case of pyrovalerone which induced a release of NE from the stored and newly synthesized NE, the effects were abolished after the pretreatment by both reserpine and α-MT.

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