Br. J. Pharmac. (1979), **67**, 323–325

THYROTROPHIN RELEASING HORMONE STIMULATES RELEASE OF [^{3}H]-DOPAMINE FROM SLICES OF RAT NUCLEUS ACCUMBENS *in vitro*

R.W. KERWIN & C.J. PYCOCK

Department of Pharmacology, Medical School, University of Bristol, Bristol BS8 1TD

Thyrotrophin releasing hormone (TRH) (25 to 100 μM) was found to stimulate the efflux of [^{3}H]-dopamine from small slices of rat nucleus accumbens, but not from similar slices of rat caudate nucleus. Uptake inhibition was not responsible for this action, since at 10 and 50 μM TRH had no effect on the ability of small slices of nucleus accumbens to accumulate radioactivity when incubated with 10^{-7} M [^{3}H]-dopamine. In addition the hormone had no effect on basal or dopamine-stimulated adenylate cyclase, nor did it displace [^{3}H]-spiperone binding, in membrane preparations from nucleus accumbens.

Introduction Thyrotrophin releasing hormone (TRH) is a putative peptide neurotransmitter which is thought to have an important role in the modulation of cerebral dopamine systems (Plotnikoff, Prange, Breese, Anderson & Wilson, 1972; Green & Grahame-Smith, 1974; Mora, Loizzo & Longo, 1976). Green, Heal, Grahame-Smith & Kelly (1976) have suggested that TRH modulates dopamine-mediated behavioural responses by a presynaptic mechanism of action, possibly by releasing dopamine. Furthermore, it has been suggested that, of the two major dopamine terminal areas in the rat brain, the mesolimbic nucleus accumbens (ACB) rather than the extrapyramidal nucleus caudatus (NC) is the site where TRH selectively modulates presynaptic dopaminergic mechanisms (Heal & Green, 1979). These conclusions are based on predominantly behavioural data. To test this hypothesis biochemically we have studied the effects of TRH on two presynaptic dopamine mechanisms: high affinity uptake and release of radiolabelled dopamine in small tissue slices from rat ACB and NC. To investigate a possible post-synaptic effect of TRH on dopamine receptors in ACB, we have also studied its effect on the dopamine-stimulated adenylate cyclase and dopamine receptor binding in membranes from this nucleus.

Methods The methods used to study the uptake and release of [^{3}H]-dopamine have been described in detail elsewhere (Kerwin & Pycock, 1979). Briefly, tissue cubes (0.2 mm) were prelabelled for 20 min in Krebs bicarbonate buffer pH 7.4, containing 10^{-7} M [^{3}H]-dopamine (9.0 Ci/mmol; Radiochemical Centre, Amersham) and superfused at 0.5 ml/min. Following a 30 min washout period, 1 ml fractions were collected every 2 min. The effect of a depolarizing stimulus (50 mM KCl) or TRH (25 to 100 μM), included in the superfusion, was studied on the efflux of radioactivity. Results are expressed as percentage rate constants, which is the amount of radioactivity released into the medium per min expressed as a percentage of total radioactivity in the tissue at the time of collection. In uptake studies, tissue slices (5 mg) were preincubated for 10 min at 37°C in Krebs Ringer bicarbonate buffer in the presence or absence of TRH (10 and 50 μM). [^{3}H]-dopamine was added to each tube (final concentration 10^{-7} M) and the reaction continued for a further 10 min. Uptake was terminated by rapid filtration through Whatman GF/B glass fibre filters. Following washing with 15 ml of ice-cold Krebs bicarbonate buffer, radioactivity remaining on the filters was determined. Combined tissue and filter blanks were determined by incubating tissue with [^{3}H]-dopamine at 0°C. Accumulation of [^{3}H]-dopamine is expressed as d min^{-1} mg^{-1} wet weight tissue/10 min.

The effect of TRH (50 μM) on basal and dopamine (20 μM)-stimulated adenylate cyclase was studied in crude membranes prepared from ACB using the method of Clement-Cormier, Parrish, Petzold, Kebabian & Greengard (1975). Results are expressed as pmol cyclic adenosine 3′,5′-monophosphate (cyclic AMP) formed/mg protein in 10 min (pmol mg^{-1} protein 10 min^{-1}). The effect of TRH (up to 1 μM) on dopamine receptor binding was assessed in purified synaptic membranes using the method of Creese, Burt & Snyder (1975). [^{3}H]-spiperone (30 Ci/mmol; Radiochemical Centre, Amersham) was used as the ligand in the concentration range 50 to 250 pmol and specific binding was defined as that displaced by an excess (0.1 μM) of (+)-butaclamol. Results are expressed as d min^{-1} bound mg^{-1} protein.

0007-1188/79/110323-03 $01.00

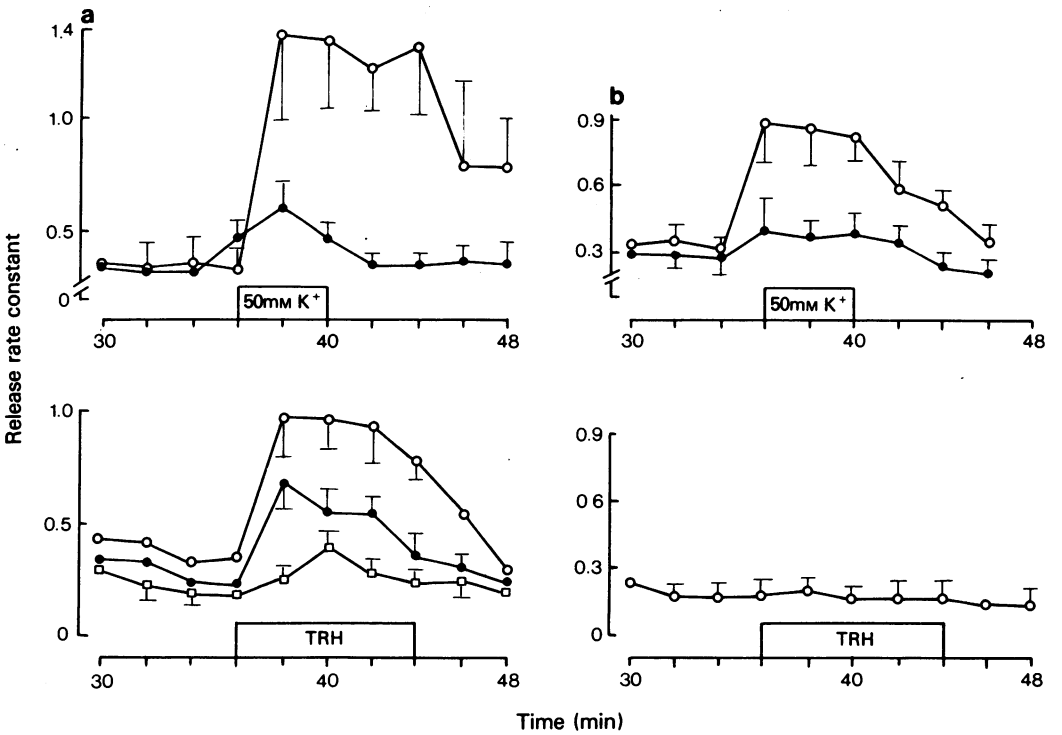

Figure 1 Upper traces: calcium-dependent, potassium-evoked (50 mM KCl) release of radioactivity from superfused slices of (a) rat nucleus accumbens, or (b) rat caudate nucleus prelabelled *in vitro* with 10^{-7} M [^{3}H]-dopamine. (○) Represent control potassium stimulation as indicated by the bar: 50 mM K^+ was added for 4 min, 36 min after the start of superfusion; (●) show experiments performed in the absence of calcium (2 mM $MgCl_2$). Results are shown as a rate constant derived from the recovered radioactivity and expressed as a percentage of the total radioactivity at the time of collection. Each point is the mean of 4 or 8 determinations. Vertical bars denote s.e. mean. Lower traces: effect of TRH on release of radioactivity from superfused slices of (a) nucleus accumbens, or (b) caudate nucleus prelabelled *in vitro* with 10^{-7} M [^{3}H]-dopamine. TRH was added for 8 min, 36 min after the start of superfusion as indicated by the bar. Doses of TRH used were 25 μM (□), 50 μM (●) and 100 μM (○).

In all experiments bacitracin (50 μg/ml) was present to inhibit peptidase activity; in uptake and release experiments, pargyline (5×10^{-7} M) was added to inhibit metabolism of [^{3}H]-dopamine and ascorbic acid (1 mg/ml) to inhibit non-specific oxidation.

Results A depolarizing stimulus (50 mM KCl) stimulated the efflux of radioactivity from slices of both ACB and NC. In both cases the effect of KCl was markedly reduced in the absence of calcium (2 mM $MgCl_2$). TRH (25 to 100 μM) stimulated the efflux of radioactivity from ACB slices but was without effect at 100 μM on release of radioactivity from NC slices. These results are shown in Figure 1. Conventional thin layer chromotographic techniques showed that radioactivity remaining in the tissue at the end of the experiment was predominantly unchanged [^{3}H]-dopamine.

At an incubating concentration of 10^{-7} M [^{3}H]-dopamine, control slices of ACB accumulated 16,300 ± 3032 d min^{-1} mg^{-1} wet wt. tissue/10 min ($n = 6$). This was not significantly different from accumulation in the presence of 10 μM TRH (18,824 ± 2272 d min^{-1} mg^{-1} wet wt./10 min, $n = 6$) or 50 μM TRH (17,888 ± 1488 d min^{-1} mg^{-1} wet wt./10 min, $n = 6$). In adenylate cyclase studies membranes from ACB synthesized 254 ± 26 pmol cyclic AMP mg^{-1} protein 10 min^{-1} (triplicate determinations, assayed in triplicate, mean ± s.e. mean pooled from two experiments, i.e. $n = 6$) which was not significantly different in the presence of TRH (50 μM; 242 ± 6 pmol mg^{-1} 10 min^{-1}). In the presence of dopamine (20 μM) the tissue increased its rate of syn-

thesis to 377 ± 34 pmol mg^{-1} 10 min^{-1}. Further addition of TRH (50 μM) was without significant effect (346 ± 9 pmol mg^{-1} 10 min^{-1}). [^{3}H]-spiperone was found to bind specifically (30% of total) and saturably to purified synaptic membranes from ACB. This was linear in the range of 50 to 250 pmol [^{3}H]-spiperone (4140 $dmin^{-1}$ mg^{-1} protein to 21,840 d min^{-1} mg^{-1} protein, quadruplicate determinations). At doses of 0.1 to 1 μM TRH no displacement of bound radioactivity was observed in this concentration range of [^{3}H]-spiperone (4500 d min^{-1} mg^{-1} protein to 21,420 d min^{-1} mg^{-1} protein, at 1 μM TRH, quadruplicate determinations).

Discussion Neurotransmitter release from nerve terminals is believed to be sensitive to calcium (Rubin, 1970) and many studies have utilized calcium-dependent potassium-evoked release of previously taken up radiolabelled transmitter to be characteristic of neurotransmitter release from intact nerve terminals (for review, see Baldessarini, 1975). Accordingly demonstration of calcium-dependent, potassium-evoked release of [^{3}H]-dopamine from rat striatal and ACB slices suggests that our superfusion system is an adequate one in which to study release processes *in vitro*. TRH stimulated the rate of efflux of [^{3}H]-dopamine from ACB slices but not from striatal slices. This increase in overflow of [^{3}H]-dopamine cannot be accounted for in terms of uptake inhibition, since at doses which stimulated [^{3}H]-dopamine release, TRH was without effect on the accumulation of [^{3}H]-dopamine into ACB slices. These data strongly support the hypothesis of Heal & Green (1979) that the behavioural effects of TRH are produced by release of dopamine from ACB rather than NC. TRH had no apparent effect on basal or dopamine-stimulated adenylate cyclase, nor did it displace [^{3}H]-spiperone binding in membranes from ACB. Thus it seems unlikely that TRH interacts directly with the dopamine receptor in ACB. It should be noted that one recent study (Costall, Hui, Metcalf & Naylor, 1979), provided no evidence for a role of TRH in dopamine-mediated behaviour. The reason for this discrepancy must remain a matter of speculation.

In conclusion, in an *in vitro* preparation, TRH seems to be able to modulate the release of dopamine from nerve terminals in ACB. Whether TRH represents a physiologically important influence on dopamine release *in vivo* is not yet known.

References

BALDESSARINI, R.J. (1975). Release of catecholamines. In *Handbook of Psychopharmacology*, Vol. 3. ed. Iversen, L., Iversen, S. & Snyder, S. New York-London: Plenum Press.

CLEMENT-CORMIER, Y.C., PARRISH, R.G., PETZOLD, G.L., KEBABAIAN, J.W. & GREENGARD, P. (1975). Characterization of a dopamine sensitive adenylate cyclase in the rat caudate nucleus. *J. Neurochem.*, **25**, 143–149.

COSTALL, B., HUI, S.-C.G., METCALF, G. & NAYLOR, R.J. (1979). A study of the changes in motor behaviour caused by TRH on intracerebral injection. *Eur. J. Pharmac.*, **53**, 143–150.

CREESE, I., BURT, D.R. & SNYDER, S.H. (1975). Dopamine receptor binding: differentiation of agonist-antagonist states with [^{3}H]-dopamine and [^{3}H]-haloperidol. *Life Sci., Oxford*, **17**, 1715–1720.

GREEN, A.R., HEAL, D.J., GRAHAME-SMITH, D.G. & KELLY, P.H. (1976). The contrasting actions of TRH and cycloheximide in altering the effects of centrally acting drugs. *Neuropharmac.*, **17**, 265–270.

GREEN, A.R. & GRAHAME-SMITH, D.G. (1974). TRH potentiates behavioural changes following increased brain 5-hydroxytryptamine accumulation in rats. *Nature, Lond.*, **251**, 524–526.

HEAL, D.J. & GREEN, A.R. (1979). Administration of thyrotrophin releasing hormone (TRH) to rats releases dopamine in n. accumbens but not in n. caudatus. *Neuropharmac.*, **18**, 23–31.

KERWIN, R.W. & PYCOCK, C.J. (1979). Specific stimulating effect of glycine on [^{3}H]-dopamine efflux from substantia nigra slices of the rat. *Eur. J. Pharmac.*, **54**, 93–98.

MORA, S., LOIZZO, A. & LONGO, V.G. (1976). Central effects of thyrotrophin-releasing factor (TRF): interaction with some antipsychotic drugs. *Pharmac. Biochem. Behav.*, **4**, 279–283.

PLOTNIKOFF, N.P., PRANGE, A.J., BREESE, G.R., ANDERSON, M.S. & WILSON, I.C. (1972). Thryotrophin releasing hormone: enhancement of dopa activity by a hypothalamic hormone. *Science, N.Y.*, **178**, 417–418.

RUBIN, R.P. (1970). The role of calcium in the release of neurotransmitter substances and other hormones. *Pharmac. Rev.*, **22**, 389–423.

(*Received June 4, 1979.*)