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RAPID COMMUNICATION

Stimulus Generalization of 1-(3-Trifluoromethylphenyl)Piperazine (TFMPP) to Propranolol, Pindolol, and Mesulergine[1]

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GLENNON, R. A., M. E. PIERSON AND J. D. McKENNEY. *Stimulus generalization of 1-(3-trifluoromethylphenyl)piperazine (TFMPP) to propranolol, pindolol, and mesulergine.* PHARMACOL BIOCHEM BEHAV **29**(1) 197–199, 1988.—Using standard operant procedures with rats trained to discriminate the serotonin (5-HT) agonist 1-(-3-trifluoromethylphenyl)piperazine (TFMPP) (0.5 mg/kg) from saline, tests of stimulus generalization and stimulus antagonism were conducted with propranolol, pindolol, and mesulergine. Neither propranolol nor mesulergine antagonized the TFMPP stimulus (pindolol was not evaluated as an antagonist). However, TFMPP-stimulus generalization occurred with all three agents. These results suggest that the TFMPP-stimulus may involve both a 5-HT_{1B} and a 5-HT_{1C} mechanism and further suggest that propranolol, pindolol, and mesulergine may be capable of acting as agonists at certain populations of serotonin receptors.

Serotonin Propranolol Pindolol Mesulergine 5-HT_{1B} 5-HT_{1C}

THE non-selective β-adrenergic antagonists propranolol and pindolol display a significant affinity for central 5-HT_1 serotonin sites. These agents display a nearly comparable affinity for 5-HT_{1A} and 5-HT_{1B} subpopulations of 5-HT sites [3, 10, 14] and constitute the only agents that have been consistently shown to behave as 5-HT_{1A} antagonists. For example, propranolol and/or pindolol antagonize (a) the serotonin syndrome [15], (b) the hypothermic effect [9], and (c) the discriminative stimulus [16] produced by the 5-HT_{1A}-selective agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH DPAT). The effect of these agents on 5-HT_{1B}-mediated behaviors has not received much attention.

1-(3-Trifluoromethylphenyl)piperazine (TFMPP) serves as a discriminative stimulus in rats [8]; furthermore, current evidence suggests that the stimulus effects of TFMPP are 5-HT_{1B}-mediated [2,11]. The TFMPP-stimulus does not generalize to the 5-HT_{1A} agonist 8-OH DPAT or to the putative 5-HT_2 agonist DOB but does generalize to agents that display a high affinity for 5-HT_{1B} sites (e.g., RU 24969, mCPP). In addition, the TFMPP-stimulus is not antagonized by 5-HT_2-selective antagonists. (See Glennon [7] for a general review of the discriminative stimulus properties of site-selective serotonin agonists.) Recently, we have found that TFMPP binds at 5-HT_{1C} sites with a significant affinity (i.e., Ki=27 and 120 nM for 5-HT_{1B} and 5-HT_{1C} sites, respectively) [14]. The ergoline derivative mesulergine possesses a very high affinity for 5-HT_{1C} sites, display greater than a 6000-fold selectivity for 5-HT_{1C} versus 5-HT_{1B} sites, and [^{3}H]mesulergine is commonly employed as a radioligand for labeling 5-HT_{1C} sites [3,10]. Thus, it was of interest to examine the effects of propranolol, pindolol and mesulergine in animals trained to discriminate TFMPP from saline.

METHOD

The animals used in this study were nine male Sprague-Dawley (225–350 g) rats. All animals were housed individually and had free access to drinking water. The animals were maintained at 80% of their free-feeding weight by partial food deprivation. The drug discrimination and stimulus generalization studies were conducted as previously described in greater detail [8] and will only be briefly outlined here. Using standard two-level operant chambers (Coulbourn Instru-

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ments, model E10-10), the animals were trained to discriminate TFMPP from saline employing a variable-interval 15-sec schedule of reinforcement for food (sweetened powdered milk) reward. That is, after lever-responding was established, each daily session was preceded by intraperitoneal administration of either TFMPP (0.5 mg/kg) or vehicle (0.9% saline). A presession injection interval of 15 min was used and the training sessions were of 15 min duration. Responding on one of the levers was reinforced after administration of TFMPP and responses on the opposite lever were reinforced after administration of saline; the right lever was designated the drug-appropriate lever for approximately half of the animals. On every fifth day, learning was assessed during an initial 2.5-min nonreinforced (extinction) session, followed by a 12.5-min training session. Data collected during the extinction sessions included responses on the drug-appropriate lever (as a percent of total responses) and response rates (responses per min). Once the animals consistently made greater than 80% of their responses on the drug-appropriate lever after administration of TFMPP, and less than 20% of their responses on this same lever after administration of saline, the stimulus generalization studies were begun. During this next phase of the study, discrimination learning was insured by continuation of the training sessions on a daily basis (except on a generalization test day; see below). On one of the two days prior to a generalization test, approximately half of the animals would receive training drug and half would receive saline; after a 2.5-min extinction period, training was continued for 12.5 min. Animals not meeting the original 80%/20% criteria were excluded from the immediately following generalization test session. During investigations of stimulus generalization, test sessions were interposed amongst the training sessions; however, after the 2.5-min extinction period, the animals were returned to their home cages. Doses of challenge drugs were administered in a random order, using a 15-min presession injection interval (except where noted otherwise), to groups of normally 5–7 rats. Stimulus generalization was said to have occurred when the animals made ≥80% of their responses on the drug-appropriate lever; animals making fewer than 5 total responses were reported as being disrupted. ED50 doses (i.e., doses at which animals would be expected to make 50% of their responses on the drug-appropriate lever) were calculated by the method of Finney [5]. In the stimulus antagonism studies, propranolol (15 min) and mesulergine (45 min) were administered prior to administration of either 0.5 mg/kg of TFMPP or 1.0 ml/kg of saline; 15 min later, the animals were tested.

Drugs

1-(3-Trifluoromethyphenyl)piperazine hydrochloride (TFMPP) was obtained from RBI (Natick, MA) and propranolol hydrochloride and pindolol from Sigma Chemical Company (St. Louis, MO). Mesulergine (CU 32-085; batch 84909) was a gift from Sandoz Ltd. (Basle, Switzerland). With the exception of pindolol, solutions of all drugs were made fresh daily in 0.9% sterile saline. Pindolol (free base) was first dissolved in one equivalent of 0.01 N hydrochloric acid before diluting with saline. All injections were via the intraperitoneal route.

RESULTS AND DISCUSSION

Administration of 0.5 mg/kg of TFMPP in combination with propranolol (2.0–12.5 mg/kg) did not result in attenuation of the TFMPP stimulus; however, at the highest nondisruptive dose (12.5 mg/kg) evaluated, only half of the animals responded and the response rate was depressed (Table 1). In the control experiments (i.e., saline in combination with propranolol) 12.5 mg/kg of propranolol administered prior to 1.0 ml/kg of saline resulted in drug-appropriate responding suggesting that propranolol might be acting as an agonist. A subsequent generalization study showed that (in

TABLE 1

RESULTS OF STIMULUS GENERALIZATION AND ANTAGONISM STUDIES USING RATS TRAINED TO DISCRIMINATE TFMPP (0.5 mg/kg) FROM SALINE*

Agent	Dose (mg/kg)	N†	Drug-Appropriate Responding (SEM)	Resp/Min (SEM)
TFMPP	0.5	9/9	94% (2)	15.5 (2.8)
Saline (ml/kg)	1.0	9/9	12% (4)	14.8 (2.1)
Propranolol + TFMPP	2.0	4/4	89% (5)	17.5 (3.9)
	5.0	4/4	91% (4)	17.8 (5.0)
	7.5	3/4	96% (4)	17.5 (8.1)
	12.0	6/6	86% (5)	9.7 (5.3)
	12.5	3/6	92% (4)	5.8 (2.2)
	20.0	1/6	—‡	
Propranolol + Saline	12.5	4/4	92% (4)	15.8 (1.3)
Propranolol	1.0	4/4	8% (3)	15.7 (1.1)
	2.0	9/9	41% (12)	15.9 (3.0)
	5.0	9/9	49% (8)	14.8 (3.3)
	9.0	8/8	70% (6)	9.2 (1.5)
	12.0	7/7	80% (7)	11.6 (1.7)
	14.0	7/7	83% (5)	16.4 (5.0)
			ED50=4.4 (2.5–7.6) mg/kg	
Pindolol	5.0	5/6	26% (12)	9.0 (1.3)
	9.0	5/6	33% (17)	14.0 (1.0)
	15.0	6/6	52% (15)	8.3 (1.0)
	20.0	7/7	60% (6)	17.7 (5.5)
	22.0	4/4	97% (3)	4.8 (0.4)
	25.0	0/5	—	
			ED50=10.6 (6.9–16.5) mg/kg	
Mesulergine + TFMPP	0.25	3/3	95% (4)	11.6 (7.7)
	0.8	5/8	71% (13)	10.6 (3.3)
	1.2	5/5	91% (5)	23.5 (4.2)
	2.0	6/6	93% (4)	19.9 (6.4)
	4.0	7/8	88% (6)	10.2 (2.6)
Mesulergine + Saline	0.6	8/9	27% (14)	13.3 (3.4)
	0.8	4/5	14% (7)	11.7 (3.2)
	1.2	3/5	30% (17)	14.4 (5.0)
	2.0	4/4	36% (11)	12.7 (1.9)
	4.0	3/4	68% (16)	14.8 (6.9)
	4.3§	5/8	80% (7)	14.1 (6.5)
			ED50=2.2 (1.0–4.8) mg/kg	

*A 15-min presession injection interval was used except that propranolol and mesulergine were administered 15 and 45 min, respectively, prior to TFMPP or saline. ED50 values are followed by 95% confidence limits.

†N=number of rats responding/number to receive drug.

‡Disruption of behavior (i.e., no responding).

§Solubility problems encountered at doses higher than 4.3 mg/kg resulted in erratic results.

the absence of post-administration of either TFMPP or saline) the TFMPP-stimulus generalized to propranolol in a dose related manner (Table 1). Because of these findings, pindolol was evaluated only as an agonist; here also, stimulus generalization occurred in a dose related manner (Table 1). Mesulergine did not antagonize the TFMPP-stimulus at doses of up to 4 mg/kg; however, in the control studies, 4.3 mg/kg of mesulergine (followed by administration of 1.0 ml/kg of saline) resulted in TFMPP-stimulus generalization. (Due to the limited supply of mesulergine, a stimulus generalization study in the absence of post-administration of saline was not conducted.)

In addition to acting as 5-HT_{1A} antagonists, the results of the present study demonstrate that propranolol and pindolol can produce stimulus effects similar to those of TFMPP. Because of the affinity, and relatively high selectivity of these agents for 5-HT_{1B} versus 5-HT_{1C} sites (e.g., pindolol binds at 5-HT_{1B} sites with a Ki value of 34 nM whereas its Ki at 5-HT_{1C} sites is >10,000 nM [14]), this is further support that the TFMPP-stimulus may involve a 5-HT_{1B}-mediated mechanism. However, due to the selectivity of mesulergine for 5-HT_{1C} versus 5-HT_{1B} sites, the data obtained with mesulergine also implicate 5-HT_{1C} involvement. It might be added, however, that none of these agents is as potent as TFMPP itself (ED50=0.17 mg/kg) [7].

It should be noted that TFMPP can release endogenous stores of 5-HT and is a weak inhibitor of 5-HT uptake and of monoamine oxidase; it is believed, however, that TFMPP acts primarily as a direct 5-HT agonist [6,13]. TFMPP also appears to have a direct effect on dopamine neurons in the substantia nigra [4] and, as such, its stimulus properties might involve a dopaminergic component. However, the TFMPP-stimulus does not generalize to either amphetamine or apomorphine, nor can it be antagonized by the dopamine antagonist haloperidol [2]. Finally, there is the possibility of a direct or indirect involvement of adrenergic mechanisms. TFMPP binds at β-adrenergic sites with low (micromolar) affinity but its affinity for α_1-sites is only an order of magnitude less than that at 5-HT_{1B} sites [12]. Mesulergine also displays a modest affinity for α_1-sites [1], whereas propranolol and pindolol are by definition non-selective β-adrenergic antagonists. Thus, although the likelihood of adrenergic involvment in the stimulus effects produced by TFMPP appears miminal, it can not be completely discounted at this time.

The present data not only shed light on the discriminative stimulus produced by TFMPP, but also suggest that propranolol, pindolol, and mesulergine (which are generally considered to be serotonin antagonists) may in fact possess agonist properties at certain populations of 5-HT receptors. Indeed, if the TFMPP-stimulus involves a 5-HT_{1C} mechanism, mesulergine may constitute the first 5-HT_{1C}-selective agonist. These results may have significant ramifications with respect to future studies involving serotonergic mechanisms in that this agonist activity will need to be taken into account.

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